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United States NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1978 - September 30, 1979

VOLUME II

INDIVIDUAL PROJECT REPORTS

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NATIONAL INSTITUTE OF MENTAL HEALTH

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NOTICE OF
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Central Amines and Psychiatric Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.57	1.27	.30

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A collaboration between the National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) involves the study of central amine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients, largely personality disorders and alcoholics. Results to date indicate that aggressive behavior may be inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with CSF 3-methoxy-4-hydroxyphenyl glycol (MHPG) and cyclic 3',5'-adenosine monophosphate (c-AMP). Alcoholics appear to have an increased 5HIAA in relation to acute intoxication. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-β-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO) and high red-cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. Within these populations neither clinical depression nor aggressive behavior appears to be related to alcoholism. These patients have also been used as control data for other psychiatric diagnostic groups at NIH.

Project Description:

Objectives: Evidence obtained in recent years indicate that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), and gamma-amino butyric acid (GABA), among others, act as central nervous system neurotransmitters and/or modulators of central nervous system (CNS) transmission. Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illnesses (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these hypotheses to improved diagnosis and treatment of the major psychiatric disorders. Relatively little direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. Recent data suggests that affective disorders and psychoses may yield to biological sub-typing which would enable a more effective choice of pharmacological therapy for individual patients. There has been virtually no data on central neurochemical function in the various personality disorders--a rather striking deficit in our knowledge, considering the evidence suggesting that some personality disorders have patterns of family history suggestive of a genetic component. The data from animals that suggests a close relationship between aggressive behavior and biogenic amines renders this a particularly interesting area for exploration. Alcoholism is also an area in which neurotransmission needs more study. The purpose of this present project would be to extend the studies of central amine turnover into larger populations of psychiatric patients including manic-depressive psychosis, schizophrenia, various personality disorders, and alcoholism.

Methods Employed: A technique has been developed at NIMH which allows for the direct estimation of brain amine turnover in psychiatric patients. This method involves the administration of probenecid, a drug which blocks the process by which the amine metabolites are removed from the brain-cerebrospinal fluid (CSF) system into the blood. Following blockade of their removal, the metabolites of the amines accumulate in the spinal fluid at a rate which is in direct proportion to their synthesis or turnover rate in the brain. Experiments with this technique at the Clinical Center of the NIH have led to the following conclusions: 1) experience in many patients has indicated that both the lumbar puncture and the administration of probenecid are safe procedures not associated with significant side effects or toxicity, except for nausea; 2) studies of this technique in conjunction with specific amine precursors and synthesis inhibitors have provided direct evidence that the probenecid induced accumulation of amine metabolites does reflect amine synthesis and turnover rates; 3) application of this technique has been shown to be capable of uncovering differences in central amine function among subgroups of patients with manic-depressive illness and other functional psychotic states.

Major Findings: About 150 patients have participated in this study to date. Preliminary findings suggest that a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment, when compared to a mixed control group from NIMH (normals and patients with non-CNS neurological disorders), have similar baseline 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenyl glycol (MHPG); however, a small group (N=8) shows a significantly elevated vanillylmandelic acid (VMA). Furthermore, results to date indicate that aggressive behavior may be inversely correlated with 5HIAA and positively correlated with MHPG. A group of personality disorders have also shown no statistically significant difference in CSF cyclic 3',5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients, though aggressive behavior is positively correlated with c-AMP. Those patients who were administratively discharged from the service and those with prior history of suicidal attempts have shown lower CSF 5HIAA and higher MHPG, c-AMP and c-GMP.

A group of alcoholics have also been studied. It has been found that alcoholics do not differ from personality disorders with respect to HVA. However, studies do indicate that the mean 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that, though HVA values do not change in relationship to the time period post intoxication-withdrawal, they are depressed concomitant with the use of disulfiram (Antabuse), a dopamine-β-hydroxylase (DBH) inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics versus normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Other studies indicate that lower DBH is correlated with increasing psychopathology as measured by the Minnesota Multiphasic Personality Inventory (MMPI) and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyl transferase (COMT) are associated with disulfiram-induced psychoses.

Other studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism. Aggressive behavior can be associated with alcoholism when the alcoholics are also personality disorders.

Significance for Mental Health Research: Central cerebral functioning is greatly under-studied in two major groups of psychiatric patients, namely personality disorders and alcoholics. Studies of animal models and hyperactive children suggest a relationship between central neurotransmitter systems and aggressive behavior. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. This project was submitted to the Navy for reapproval in March 1979. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00021-14 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on Sleep

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental findings over the past decade promise understanding of the basic biochemical process underlying sleep and the increasingly clear demonstrations of severe sleep disturbances associated with psychiatric disorders, as well as the existence of sleep disorders themselves, indicating need for better means of alleviation. To the extent that the process of sleep can be understood, more effective treatment of disorders of sleep will surely follow. In addition to the clinical, biochemical and endocrinological work this laboratory has done in the past, we are beginning to ask fundamental questions about the nature of sleep, using chemical and psychological probes.

These studies contribute to the extensive national and international efforts to understand why we spend one-third of our lives in this state.

In addition, the Unit is actively engaged in studying the effects of pharmacological agents on human memory, both in normals and in patients with learning disorders.

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Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep, and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbance will surely follow.

I. Major Findings: Pharmacological Studies of Sleep

A. Cholinergic

1. Relationship of Cholinergic Activity to REM Narcoleptic Attacks

In order to explore the possible relationship between levels of cholinergic activity and REM narcoleptic attacks, we have begun therapeutic trials of the cholinergic blocking agent, Artane (trihexyphenidyl 2 mg tid). Initial results from narcoleptic patients were encouraging in that all showed clinical improvement, especially in terms of less frequent attacks of cataplexy, but tolerance appeared to develop. The converse procedure of administering physostigmine (0.5 mg IV q 5 min. to a total dose of 1.5-2.5 mg), an inhibitor of cholinesterase, in an effort to determine whether increased cholinergic activity induces REM narcoleptic cataplexy did not have any discernible effects. In addition, physostigmine had no apparent effects on the mood of these patients, contrary to recent reports involving psychiatric patients.

2. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

Physostigmine induces REM sleep or arousal depending upon the dose and time of administration. Thirty-three normal volunteers received 40 physostigmine (ph 0.5 mg over 2.3 min) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 min after sleep onset reduced time from infusion to onset of REM from 78.9 ± 14 min (sal) to 52.4 ± 10.4 (phy) ($p<0.05$, two-tailed "t" test). (2) Infusions given 35 min after sleep onset reduced time from infusion to onset of REM from 65.7 ± 15.2 (sal) to 11.2 ± 2.1 (phy) ($p<0.005$). REM was induced more rapidly by physostigmine infusions given 35 min after than 5 min after sleep onset ($p<0.01$). (3) For the infusions at REM onset, 5 min after and 25 min after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 min after > 25 min after REM. Although a dose of 0.5 mg awoke the subjects when given 25 minutes after the first REM period, a dose of 0.25 mg induced REM.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first REM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second NREM period produces different intensities of arousal.

Using single bolus infusions of physostigmine administered at different times during the first and second NREM periods, we have recently shown that REM sleep and arousal can be induced in a time- and dose-dependent manner. In view of the extremely short-lived action of a bolus infusion of physostigmine we have hitherto not been able to determine if the duration of a REM period can be prolonged in addition to triggering its onset.

Seven normal male volunteers (Mean age 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pretreated with 0.75 mg IM methscopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset.

Physostigmine significantly decreased both the latency of the first REM period (Placebo = 108.4 ± 16.2 min (mean ± S.E.) physo = 61.7 ± 2.2 min, $p<.05$ and the duration of the second NREM period (placebo - 110.4 ± 13.6, physo - 74.4 ± 6.5, $p<.05$). The duration of individual REM periods was not altered. Although total REM time and REM per cent showed a non-significant increase of physostigmine (RT = 98.2 ± 6.4 min, R% = 25 ± 1 5%) as compared to placebo nights (RT - 80.9 ± 9.4, R% = 20.1 ± 1.9%) this can be accounted for by the greater number of REM periods on physostigmine (4.3 ± 0.3) than on placebo (3.6 ± 0.2) conditions. Cholinergic mechanisms may modulate the "timing" rather than the "maintenance" of REM.

3. Physostigmine-induced REM is associated with normal dreaming

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age 23) after pretreatment with methscopolamine 0.5 mg IM slept with an IV needle and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 minutes after sleep onset and were awakened 20 minutes after infusion. Experiment II includes awakening either 7 or 20 min following an infusion given 35 min after sleep onset; in addition subjects were instructed to go back to sleep after the 20 min awakening and were then awakened approximately 7 min after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep prior to awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire. A dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

Comparison of Dream Reports from Physostigmine-induced and Spontaneous First REM Period

		Physostigmine-induced REM Periods	Spontaneous REM Periods	p
1)	No. of REM awakenings	9	10	NS
2)	No. of dream reports*	8	7	NS
3)	Duration of REM prior to waking (mean ± SEM min)	6.6 ± 0.6	7.1 ± 0.4	NS
4)	REM Density (0-8 scale)	1.7 ± 0.2	1.7 ± 0.4	NS
5)	Vividness of dreams*	3.1 ± 0.4	2.7 ± 0.4	NS
6)	Unusualness (0-5)*	1.6 ± 0.5	1.7 ± 0.3	NS
7)	Emotionality (0-5)*	2 ± 0.4	1.9 ± 0.5	NS

* These scores were obtained from a questionnaire filled out independently by both subjects and experimenter and from a structured interview conducted by the experimenter.

During Experiment I and the 7 minute post-infusion awakenings of Experiment II neither REM sleep nor dreaming was induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

4. Induction of REM Sleep in Man by Arecoline, a Cholinergic Muscarinic Agonist: Blockade by Scopolamine.

Ten normal volunteers (8M, 2F, mean age 25.3) were each studied on 4 randomly ordered experimental nights (after a night of adaptation).

After pretreatment with methscopolamine 0.5 IM on three of the experimental nights, they each received one intravenous infusion about 32 to 35 min after sleep onset of either (1) placebo, (2) arecoline 1.0 mg IV, or (3) arecoline 1.5 mg IV. On the fourth night pretreatment was scopolamine 0.5 mg IM followed by IV infusion of arecoline 1.5 mg. The results were: (1) Both arecoline 1.0 mg and 1.5 mg triggered the onset of REM sleep, 20.1 and 15.9 min after infusion, respectively. (2) Pretreatment with scopolamine blocked the REM-inducing effect of arecoline 1.5 mg IV. (3) Once the onset of the first REM period was moved forward subsequent nonREM-REM cycles were advanced without altering REM-REM intervals. (4) The number of REM periods was significantly increased after arecoline 1.0 mg (4.1±0.3) and 1.5 mg (4.5±0.2) compared to placebo (3.5±0.2). (5) Total REM time and percent after arecoline 1.5 mg (100.7±6.4 min, 24.7±1.7%) was significantly greater than placebo (76.2±5.4 min, 18.1±0.9%). The data with arecoline, a specific cholinergic muscarinic agonist, confirms our previous findings with physostigmine and suggests that acetylcholine may play a role in the triggering of REM sleep in man.

5. Experimental Shortening of REM Sleep Ultradian Rhythm with Timed Multiple Infusions of Arecoline.

Seven normal volunteers (4M, 3F, mean age 25 yrs) were studied under the following two experimental conditions. (1) Pretreatment with methscopolamine 0.5 mg IM followed by three IV infusions of arecoline (1.5 mg, 1.0 mg and 1.0 mg doses) given at the following three times respectively: (a) 35 min after sleep onset; (b) 30 min after end of first REM period; (c) 30 min after end of second REM period. (2) Pretreatment with methscopolamine followed by three placebo (saline) IV infusions. Subjects were run either in pairs or groups of 3 such that the timing of the placebo infusions was "yoked" to the arecoline infusions.

TABLE: Duration (minutes) of NREM and REM periods (mean ± SEM)

	NREM$_1$	REM$_1$	NREM$_2$	REM$_2$	NREM$_3$	REM$_3$	NREM$_4$	REM$_4$	NREM$_5$	REM$_5$
Placebo	95.7 ±14.7	14.1 ±3.9	89.1 ±5.7	22.8 ±3.2	78.4 ±4.6	25.5 ±4.7	-	-	-	-
Arecoline	42.2* ±2.6	10.3 ±2.4	38.3* ±1.3	12.8* ±2.5	41.4* ±3.7	21.0 ±4.9	84.7 ±4.1	22.1 ±5.4	65.4 ±4.6	28.8 ±5.0
								(N=6)	(N=6)	

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*p<.01

The results of this study are: (1) It was possible to transform a normal REM ultradian rhythm (with REM-REM interval of about 100 min) into a rhythm with REM$_1$-REM$_2$ interval of 48.6 min and REM$_2$-REM$_3$ interval of 54.2 min. (2) There were a significantly greater number of REM periods after arecoline (5±0.22) than placebo (3.71±0.29) resulting in greater REM time and percent (arecoline = 104.1±1.5.6 min, 24.8±1.9% and placebo = 84.4±4.6 min, 20.1±1.2%). (3) Duration of individual REM periods were either unchanged or shortened (e.g., REM$_2$). Acetylcholine plays a role in the timing of REM sleep. The increase in REM time seen appears to result from an increase in the number but not the duration of REM periods. There also appears to be a "ceiling" after which REM time cannot be further increased by cholinergic agents since both single and multiple infusions of arecoline produce the same amount of increase in REM time.

6. Acetylcholine and Sleep: Effect of Oral Choline on Normal Human Sleep.

Choline, a normal dietary constituent, has recently been shown to be a precursor of brain acetylcholine. If so, in the light of our previous work with physostigmine and arecoline we would predict that choline administered to humans may alter REM sleep parameters. A double blind crossover design with 12 normal male subjects was used. Subjects slept for 3 nights (1 adaptation and 2 experimental) and just prior to "lights out" (11:30 p.m.) were given an elixir containing either 10 g of choline chloride or placebo matched for taste, color and consistency.

Effects of Choline Chloride (10 gm) or Placebo

Upon Sleep of Normal Volunteers (N=12)

	Placebo	Choline (10 gm)
Total Recording Period	472 ± 5*	492 ± 4
Sleep Latency	33 ± 8	24 ± 4
Total Sleep	422 ± 8	446 ± 6
Non-REM	326 ± 10	347 ± 8
Stage I	12 ± 3	10 ± 3
Stage II	269 ± 7	273 ± 9
Stage III	22 ± 4	29 ± 4
Stage IV	25 ± 5	33 ± 5
Stage V	25 ± 5	33 ± 5
REM Sleep	95 ± 6	100 ± 5
REM Latency	92 ± 12	107 ± 12
Intermittent Awake	13 ± 4	17 ± 5
Early Morning Awake	4 ± 1	3 ± 1

* mean ± SEM
values are in minutes

The results were: (1) Choline did not have any effect on conventional sleep parameters when data from all 12 subjects were analyzed. (2) When the subjects were rank ordered with respect to their placebo (baseline)

REM latency, REM time and REM %, we found that low placebo REM latencies were increased and high placebo REM latencies were decreased by choline. The correlation between placebo (baseline) REM latency and the change in REM latency after choline (i.e., choline-placebo value) was inversely correlated $r=-0.90$, $N=12$ ($p<.01$). Similarly there was an inverse correlation between placebo REM time and REM % and change after choline ($r=0.63$ for REM time and $r=0.61$ for REM%, both $p<.05$). Whether the above changes in REM parameters produced by choline represents a genuine central neuromodulatory effect or is no more than a statistical artifact is unclear. Further experiments to test this hypothesis as well as the effect of intravenous choline chloride administered during sleep in normal volunteers is currently underway and will be discussed at the meeting.

7. The Effect of Piperidine, a Cholinergic Nicotinic Agent on Normal Human Sleep.

Piperidine is an alicyclic amine with nicotine-like cholinomimetic effects on central and peripheral nervous system. In view of conflicting reports on the effect of piperidine on REM Sleep in rodents, we undertook to study the effect of oral piperidine HCl in normal volunteers. Using a 4x4 Latin square design, 8 subjects were recorded on 4 nights under the following four experimental conditions (a) placebo, (b) piperidine 200 mg, (c) piperidine 400 mg and (d) piperidine 600 mg. All medications were given prior to "lights out" (11:30 p.m.). No changes in any sleep parameter including REM latency, REM time and REM density were observed after piperidine. Although data from animal pharmacology suggests that the doses used in our study would have central effects, the possibility still remains that physiologically effective doses were not used. Intravenous administration of piperidine during sleep is also currently underway.

8. Scopolamine-induced Muscarinic Supersensitivity in Normal Man: Changes in Sleep

An injection of scopolamine ($6\mu g/kg$) was administered on 3 consecutive mornings to normal human subjects. Sleep recordings obtained at night (when the central anticholinergic effect of the morning scopolamine was no longer present) indicated a significant reduction in latency to REM sleep onset on the nights following the second and third injection. REM latency was significantly reduced on the second (50 min ± 12) and third (45 min ± 13) scopolamine nights as compared with baseline (96 min ± 16) ($p<.005$). This effect is opposite to the direct pharmacological action of nighttime administration of scopolamine (i.e., prolongation of REM latency). Furthermore, scopolamine pretreatment on 2 consecutive mornings also potentiated the REM-inducing effect of arecoline, a central muscarinic agonist. The time from infusion to REM was 17 min ± 9 after scopolamine pretreatment and 47 min ± 11 after saline ($p<.05$). These data are consistent with development of cholinergic supersensitivity following cholinergic blockade.

9. Identification of a Stimulatory Cholinergic Mechanism in Sleep-related Growth Hormone Secretion.

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean ± SEM overall GH level of 2.89 ± 0.44 and the mean peak GH level of 11.09 ± 3.11 ng/ml were dramatically reduced to 0.75 ± 0.04 and 1.04 ± 0.25 ng/ml, respectively ($p<0.0001$ and $p<0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep (12.1 ± 2.6% control vs. 10.3 ± 2.5% drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

10. Piperidine Enhances Sleep-related and Insulin-related Growth Hormone Secretion

Piperidine (100 mg) and saline control infusions were administered for 30 minutes beginning at sleep onset to 8 normal volunteers. Piperidine significantly enhanced sleep-related growth hormone secretion but had no effect on sleep-related prolactin secretion. Piperidine had no effect on growth hormone when infused during the daytime, but significantly increased insulin induced growth hormone release in normal controls. These data are consistent with the view that cholinergic pathways facilitates sleep-related and insulin-induced growth hormone secretion.

11. Choline Chloride Reduces Insulin-induced Growth Hormone Secretion without Affecting Sleep-related Growth Hormone

Choline chloride (10 gm) or placebo were administered one hour prior to testing the effect of insulin-induced growth hormone release or prior to bedtime. Choline significantly reduced the insulin-induced growth hormone peak from 24 ± 4 to 17 ± 4 ng/ml ($p<.01$). No effect on nocturnal growth hormone or prolactin release was noted. The mechanism by which choline affected insulin-induced growth hormone is not clear, but probably does not result from increased cerebral acetylcholine synthesis.

B. Histamine: Effects of L-Histidine on Sleep.

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electroencephalographic sleep patterns. L-histidine was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days. No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

C. Catecholamines

1. Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double-blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithium carbonate treatment.

2. Pimozide Attenuates d-Amphetamine-induced Sleep Changes in Man

Pretreatment with pimozide (mean dose=13 mg/day) blocked the effect of d-amphetamine (20 mg base, administered by intravenous bolus infusion at

0815) on all-night EEG sleep patterns in seven hospitalized psychiatric patients. Each patient was studied for five nights (2 nights baseline, 1 night on the day of the infusion, and 2 nights recovery) with and without pretreatment with pimozide. Without treatment with pimozide, d-amphetamine significantly reduced duration of total sleep, REM and nonREM sleep, Stage I, and Stage II. With coadministration of pimozide, d-amphetamine had no effect on sleep. These results suggest that the d-amphetamine induced changes in sleep are mediated by dopaminergic neurons.

D. Serotonin:

1. Relationship to Narcolepsy.

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

2. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet.

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique-the acute administration of a tryptophan-free amino acid diet.

Prior to the sleep study we confirmed the efficacy of this method in a biochemical study on 24 rats. They were trained for a 20-day period to eat their entire daily food supply (an amino acid diet) in 2 hours. On the twenty-first day they were divided into two groups who received the same diet or one of similar composition but lacking tryptophan. Animals from each group were then decapitated at intervals over the next 24 hours. At the end of the first two-hour feeding, serotonin and 5-HIAA concentrations had dropped significantly. They continued to decrease until they reached a nadir eight hours after the start of feeding, at which time serotonin and 5-HIAA levels were 30 and 41 percent of control values, respectively. At 24 hours they had started to increase but were still significantly less than control values.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p<.05$), and there was a small but significant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

	Control Diet	Tryptophan-Free Diet	Statistical Significance
Total sleep	519.6±25.7*	529.8±27.0	NS
Sleep latency	45.5±10.6*	35.8±10.6	NS
Intermittent waking (IW/TRP)	33.9± 3.0	30.8± 2.2	NS
nonREM sleep (NR/TST)	87.6± 2.4	91.4± 2.3	$p<0.05$
REM sleep (REM/TST)	12.3± 2.4	8.5± 2.2	$p<0.05$

Values represent mean number of 30 sec. epochs ± SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

Hours after food presentation	Control diet		Tryptophan-free diet	
	Serotonin	5HIAA	Serotonin	5HIAA
2	1.43±0.04	0.52±0.06	0.80±0.02***	0.38±0.02**
4			0.62±0.01***	0.27±0.02***
8			0.44±0.02***	0.21±0.01***
24	1.52±0.02	0.56±0.04	0.65±0.02***	0.41±0.04*

Each value represents the mean from four rats ± S.E.M., in units of μg/g brain tissue.

* $p<0.05$ by t-test when compared to mean of combined control values.
** $p<0.01$
*** $p<0.001$

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

3. Intravenous Chlorimipramine Affects REM Cycle in Patients with Excessive Daytime Sleepiness and Control Subjects

The sleep of 5 normal volunteers and 4 patients with excessive daytime sleepiness was studied following intravenous administration of 5 mg chlorimipramine during the first period of rapid eye movement (REM) sleep. There was a 95% increase in the duration of the first REM cycle (time lapsed from onset of first REM period to the onset of the second REM period) in all 9 subjects, $p<.01$, compared to saline control nights. Other effects included a significant, $p<.05$, mean decrease in the post-infusion REM time and REM percentage to approximately one half of the control levels. Phasic events associated with REM sleep were also diminished during the post-infusion period with significant mean decreases in the REM density and the REM index per minute of sleep.

E. Ethanol:

1. A Dose-response Study of the Acute Effects of Ethanol on the Sleep of Rats.

Although there is no shortage of studies on the effects of ethanol on sleep of animals, there is little dose-response data available. In order to examine this relationship more carefully, we have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. By dividing the recording into sequential 3.5 hour periods, it became clear that this effect occurred most strongly in the first 3.5 hours of sleep. Although the number of awakenings was not changed over the recording as a whole, they were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

2. Hypnotics and Ethanol: Residual EEG Effects.

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity.
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p<0.02$ and $p<0.05$ respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p<0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following triazolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

3. Sleep, Behavior, and Blood Ethanol Concentration in the Ethanol-Dependent Rat.

In this study sleep recordings were performed to further characterize a previously-described animal model of ethanol dependence. In summary, this consists of administering approximately 10 gm/kg of ethanol per day by intragastric intubation in six divided doses for four days. Eight-hour EEG recordings were made during the baseline period and the first and third days of ethanol administration (induction period). One group of animals (N=14) was also recorded on the first and fourth withdrawal days; a second group (N=5) was recorded on days 1-4 and 6 of withdrawal. During the induction period, REM sleep time was decreased, particularly on the third ethanol day when blood ethanol concentrations were highest.

This largely reflected decreases in the number of REM sleep episodes. The first day of ethanol withdrawal was associated with a decrease in total sleep time, primarily due to loss of nonREM sleep. Total sleep time gradually returned to normal, and in fact slightly exceeded baseline levels on the third withdrawal day. At this time, REM percentage increased to twice the baseline values. This was related to a decrease in REM latency and an increase in the number of REM sleep episodes. REM percentage then decreased such that on the fourth withdrawal day it approached baseline values. Total sleep time and other sleep parameters were also indistinguishable from baseline on the fourth day. Blood ethanol concentrations on the first withdrawal day were inversely related to behavioral scores of withdrawal and to EEG sleep latency.

The major observations in this model seem consistent with observations in the literature on ethanol withdrawal in animals and humans. Several of the EEG parameters--notably the initial decreased sleep and later REM rebound--may be useful in testing new treatments for ethanol dependence.

F. Enzymes:

1. The Relation of Platelet MAO to Human Sleep.

Platelet monoamine oxidase (MAO) activity has been related to likelihood of psychiatric contact, scores on personality tests, family history of suicide, and other measures. Most studies have shown decreased activity in chronic schizophrenics and bipolar depressed patients. In the present study, we have examined the sleep of normal volunteers with high and low platelet MAO activity.

These volunteers were drawn from groups reported elsewhere, obtained by selecting individuals with MAO activity in the highest and lowest decile of a population of 375 students. Ten high [mean 16.1±3.2 (sd) nanomoles per 10^8 platelets per hour] and ten low [7.1±2.3 (sd)] volunteers, 5 men and 5 women in each group returned for sleep studies. Each subject was recorded for two nights following two adjustment nights.

The group with high platelet MAO activity had significantly lower REM sleep time (69.9±5.2 vs 92.7±8.6 minutes; $p<0.05$), lower REM density 1.5±0.1 vs 1.9±0.1, and presumably as a result of the previous two factors, a lower REM index, a measure of the total number of eye movements (105.5±9.3 vs 184.8±21.8; $p<0.01$). All other sleep variables were similar for the two groups.

An examination of the REM sleep parameters in which the two groups differed suggests that the values for the low platelet MAO group are similar to those of other volunteers we have studied. An implication, then, is that subjects with high platelet MAO may have less REM time and a lower REM density than might be expected in a general population. These observations may also constitute the first report relating individual differences in an enzyme involved in neurotransmitter metabolism to human sleep EEG parameters.

G. Arginine Vasotocin Alters REM Sleep in the Rat

In a dose-response study of intraperitoneal administration of arginine vasotocin in the rat, (1) 0.5 μg/kg had no effect, (2) 50 μg/kg reduced REM latency and REM efficiency, and induced a quiet waking state without neurological impairment, and (3) 500 μg/kg produced fatal pulmonary congestion.

II. Hormones:

A. Regulation of Sleep-Related Growth Hormone and Prolactin Secretion.

The study of pharmacologic manipulation of sleep-related growth hormone (GH) and prolactin (PRL) secretion has provided data on the roles of the serotonergic, noradrenergic and cholinergic systems in anterior pituitary regulation. The authors will review work in this area and report on two studies from their laboratory. In the first, the serotonin receptor blocker methysergide (2 mg PO q 6 h) was administered for 48 hours to normal young adults, and blood samples were drawn every 20 minutes during eight-hour sleep recordings. Methysergide administration was associated with an increase in several measures of sleep-related GH secretion. Mean peak concentrations of GH rose from 7.86±1.35 (SEM) ng/ml on control nights to 10.30±1.39 ng/ml with methysergide ($p<0.03$). Mean nocturnal PRL concentrations were decreased by 70.3%. In contrast to the increases in sleep-related GH secretion, daytime insulin-stimulated GH secretion was decreased 36.4% by methysergide.

These studies would seem to imply that: (1) serotonergic mechanisms may have an inhibitory role, and cholinergic mechanisms may have a facilitory role, in regulation of sleep-related GH secretion; and (2) data drawn from pharmacologic manipulation of daytime insulin provocative testing may lead to erroneous conclusions regarding physiologic control of GH secretion.

B. The Effect of Dexamethasone on Sleep-Related Growth Hormone Secretion in Congenital Adrenal Hyperplasia: Therapeutic Implications.

Treatment of children with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) with steroids has two goals: replacement of inadequate secretion of cortisol (and sometimes aldosterone) and suppression of oversecretion of adrenal androgens. In adults, and adolescents who have achieved full growth, it is most desirable to use long-acting steroids such as dexamethasone (DEX), given at midnight. The effects of DEX on nocturnal GH secretion needs to be evaluated, however, particularly before use in children and adolescents who have not achieved full stature. For this reason we have examined GH secretion and other endocrine measures during sleep in CAH patients receiving DEX in two different regimens.

The four females and two males who participated in the study ranged in age from 13 to 22. All but two (who were female) were salt losers. All patients received 0.5 mg DEX for six days each at 0700 and 2300 hours. Sleep studies were performed on night 5, and during a study in which several blood samples were drawn on night 6.

Examination of 24 hour excretion of 17 ketosteroids and pregenanetriol showed adequate suppression under both regimens in four of the six patients. In two subjects there was inadequate suppression with either regimen. LH was found to be secreted episodically throughout the night in a similar and normal manner with both regimens. Mean nocturnal FSH concentrations were also similar.

Examination of sleep-related GH revealed increased secretion during the first two hours of sleep when DEX was given at 0700 compared with administration at 2300. The areas under the curves of GH plots during this time were 1737±744 (SEM) and 666±359 ng/ml/min respectively ($p<0.05$). Following administration at 2300, there was a tendency for increased GH secretion later at night, which did not, however, reach significance.

Only two significant differences were noted on sleep EEG recordings made during the blood study: Following 0700 administration, there was a longer sleep latency (65.8±18.1 vs 24.2±7.9 minutes; $p<0.02$) and decreased nonREM sleep (266.8±34.4 vs 363.3± 13.7 minutes, $p<0.02$). These observations were not borne out by the recording on night 5, and hence are of questionable biological importance. The possibility remains, of course, that DEX affects sleep differently when patients undergo the slight stress of an intravenous study compared to when they do not.

These observations suggest that administration of DEX at 0700 suppresses the adrenals yet permits sleep-related secretion of GH. In contrast, administration at 2300 suppresses sleep-related secretion of GH. Further studies of chronic use, and considerations of possible effects on somatomedins, will be necessary to further establish the safe use of DEX in children and adolescents.

C. LH-RH Administration and Human Sleep.

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide of hypothalamic origin, stimulates release of LH and FSH from the pituitary; in addition it may possess direct actions on the central nervous system. It has been shown, for instance, to cause mating behavior in estrogen-primed ovariectomized rats. Its administration has been reported to produce sexual arousal in human males with hypogonadism well before androgen levels reach the normal range. Because of these implications that LH-RH may possess central effects, and since gonadal hormones have been reported to influence sleep, we have explored the possibility that LH-RH administration may influence normal human sleep.

Subjects were 5 male and 5 female young adult normal volunteers. Females were studied during the follicular phase of the menstrual cycle. Following an adjustment night, each had sleep studies on two nights. On one night they received 100 μg of LH-RH in 500 cc of normal saline intravenously over an 8 hour period starting when the lights were turned out at 11:00 p.m. On the other, randomly-sequenced night they received an equivalent volume of normal saline. The dose of LH-RH, while lower than those reported to produce human sexual arousal, has been shown to acutely raise serum LH and FSH.

Sleep EEG data revealed that LH-RH administration produced no change in the total minutes or percentage of the sleep stages, total sleep, or sleep latency. The subjects were also divided into two groups by sex, and once again there were no significant changes in sleep. It is concluded that low doses of LH-RH, sufficient to stimulate gonadal hormones, have no acute effects on normal human sleep. The possibility remains, of course, that higher doses or more potent analogues of LH-RH may have such actions.

III. Major Findings: Clinical Disorders

A. Successful Separation of Depressed, Normal, and Insomniac Subjects by EEG Sleep Data

Data from all-night EEG sleep studies were used to distinguish normal subjects, primary depressed patients, and primary insomniac patients. In part 1, we compared 41 normal subjects, 56 depressed patients, and 18 insomniacs. In a univariate comparison with normal subjects, depressed patients showed less total sleep, longer sleep latency, more early morning awake time, more intermittent awake time, less delta sleep, less sleep efficiency, and shorter rapid eye movement (REM) latencies; compared with insomniacs, depressed patients showed greater early morning awake time, shorter REM latency, greater REM index, and greater REM density. Using multivariate discriminant analysis, 82% of the sample were correctly classified by diagnosis: 100% of the normal subjects, 72% of the depressed patients, and 77% of the insomniacs. Eight variables contributed to the multivariate separation of depressed individuals from insomniacs and normals: total sleep time, total recording period, sleep efficiency, sleep latency, early morning awake time, awake time, REM time, and REM %. When the discriminant functions were applied to a second group of 18 primary depressed patients, 82% were correctly classified as depressed. These results suggest that primary depressed patients and primary insomniac patients may show relatively characteristic patterns of sleep abnormality.

Part I: Classification of Normal Subjects,
Depressed Patients, and Insomniac Patients by
Discriminant Analysis: All 12 Sleep Variables

	No. (%)		
	Normal	Depressed	Insomniacs
Normal (N=41)	41 (100)	0 (0)	0 (0)
Depressed (N=56)	13 (23.2)	41 (73.2)	2 (3.6)
Insomniac (N-18)	4 (22.2)	0 (0)	14 (77.8)

B. The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time.

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours.

Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Next, they were subdivided according to time of occurrence (i.e., which nursing duty shift they occurred in; 11 p.m. to 7 a.m., 7 a.m. to 3 p.m. or 3 p.m. to 11 p.m.). Average sleep time as estimated by nursing staff for five days before (Day -5 to -1) and five days after the day of switch (Day 0 to +4) was calculated. The day of switch was designated as Day 0.

Both D→M and M→D switches occur predominantly in the morning (7 a.m. - 3 p.m.). Among the D→M group, patients who switch at night (11 p.m. - 7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m. - 3 p.m.) ($p<0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Mrs. J was 41 when she was first admitted to the NIH Clinical Center in 1962 and was hospitalized several times over the course of two years. During her inpatient stay she was studied intensively; the nursing research team rated her every two hours on a 15 point mania scale. Checks were made every fifteen minutes during the night to determine whether she was asleep. We defined a "switch" into or out of mania as a change of manic ratings of at least three points.

Next we tried to localize the specific two-hour time period in which a switch took place using nursing ratings. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with df=11). Switches during sleep: among the 80 switches into mania 37 occurred out of sleep (41%); among the 64 switches out of mania, 27 were associated with sleep (42%).

C. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amitriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

D. Muscarinic Supersensitivity: A Possible Model for the Sleep Disturbance of Primary Depression

The sleep changes induced in eleven normal volunteers following the administration of scopolamine on 3 consecutive mornings resembles many of the abnormalities observed in the sleep of patients with primary depression: increased sleep latency, and reduced REM latency, total sleep time, and sleep efficiency. Furthermore, multivariate discriminant analysis, previously shown to distinguish the sleep records of depressed patients from those of normal controls and insomniac patients, selected the records from baseline nights as normal and those after scopolamine as predominantly depressed.

	Baseline	Scopolamine
Normal	11	4
Depressed or Insomniac	0	7+

$p < .005$ Fishers Exact Probability Test

+ depressed 6
insomniac 1

These observations suggest that muscarinic supersensitivity in normals may function as a pharmacological model for the sleep disturbances of depression.

E. Cholinergic Supersensitivity in Affective Disorders

Eleven euthymic patients with a history of primary effective disorder (4M, 7F, age 28 ± 2; 10 Bipolar, 1 Unipolar) were compared with 13 age- and sex-matched controls. Infusions of placebo, arecoline 0.5 or 1.0 mg were given 25 minutes after the end of the first REM period. Both doses of arecoline induced REM faster in patients than controls (for 0.5 mg: patients = 11 ± 2 min; controls = 38 ± 9, $p < .01$; for 1.0 mg: patients = 7 ± 1 min, controls = 19 ± 4 min). Placebo did not differentiate. Similar findings occur in depressed patients. These results suggest that state-independent cholinergic super-sensitivity may be a part of affective illness.

F. Catecholamine depletion: Sleep and Mood Changes in Man Mimic the Bipolar Switch Process

Alpha-methyl-para-tyrosine (AMPT, 3 gm/d) was administered in a double-blind fashion to 9 normal volunteers (mean age 24) for 3 days. During AMPT administration, such ratings of mood indicated significantly increased depression, while sleep studies indicated hypersomnia and reduced REM sleep. During withdrawal from AMPT, mood ratings suggested hypomania and sleep studies indicated a transient hyposomnia. Co-administration of lithium (900-1200 mg/day) attenuated the withdrawal hypomania. These results suggest that AMPT in normals mimics the bipolar "switch" process from depression to mania.

G. Sleep of Bipolar and Unipolar Depressed Patients Compared

The sleep of 36 normals, 36 unipolar patients, and 22 bipolar patients were compared. The only sleep variable which significantly differed between unipolar and bipolar patients was REM efficiency. Bipolar patients showed a significant reduction compared with unipolar patients (82% ± 3 versus 91 ± 1, $p < .001$). In addition, "hypersomnia" (defined as more sleep than the age-corrected normal mean) was more prevalent among the bipolars than the unipolars ($p < .05$, $X^2 = 3.81$).

H. Schizophrenia:

1. Schizophrenia

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

2. 5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-60000 mg/24 hr with MK486 in 18 chronic schizophrenic patients. EEG sleep studies revealed:

(a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

I. Absence of Fast Ocular Activity During REM Sleep in Familial Cerebellar Degeneration

The major sleep abnormality in a family with a hereditary cerebeller degeneration was an absolute loss of fast ocular activity during REM. The more affected the individual was with the disorder, the less likely he or she was to display fast ocular movements during REM sleep.

In the absence of neuropathological evidence, it is impossible to precisely characterize the neurologic deficit in this disorder, and, thus, perhaps provide a neuroanatomical basis for the REM's of REM sleep.

Clinically, the disease appears to involve the cerebellum predominately. It is known, however, from animal studies that cerebellectomy does not affect the REM's of REM sleep.

Although vertigo was not a prominent symptom in any of these patients, the electronystagmography results suggest a central vestibular lesion in three patients. While this data is only suggestive, it is consistent with the hypothesis put forward by Pompeiano that REM's depend upon the integrity of the medial and descending vestibular nuclei (the intellectual relevance to the absence of REM's) since Feinberg has reported diminished REM's in senile dementia.

J. A Case of Sleepwalking and Somnambulistic Homocide Associated with Psychotropic Drugs

The patient is a 44 year-old white female who had a history of one sleepwalking episode in adolescence but none after that until age 40. She also had had enuresis until age 16. Except for brief psychiatric treatment at age 16 for enuresis and anxiety, she had no psychiatric history until age 39, when she developed a florid psychosis with persecutory symptoms. She was diagnosed as schizophrenic, hospitalized for 2 weeks, and treated with phenothiazines. A year later she experienced a return of symptoms and resumed treatment on outpatient basis. Because of insomnia, she received thioridazine and Triclos (a chloral derivative) at bedtime. At 3 a.m. on the third night of this regimen, she arose from her bed and stabbed her daughter to death, possibly while asleep or in a confused state. She was found to be hallucinating and delusional when examined the next morning and diagnosed as suffering from paranoid schizophrenia. At her trial she was found innocent on the grounds of insanity and hospitalized under court order.

On phenothiazines and psychotherapy, her psychosis slowly cleared. However, she was noted to sleepwalk 2-4 times per week. Physical investigations, which eventually included EEG's with nasal-pharyngeal leads, a CAT scan, and a pneumoencephalogram, were all normal. Dilantin and tricyclic antidepressants had no effect on her sleepwalking.

After 4 years in the hospital, she was transferred to our unit, and thioridazine 300 mg q.h.s. was discontinued. During a one-month drug-free period, 11 all-night sleep EEG's along with continuous video monitoring were carried out. There were no sleepwalking episodes. However, when treated with thioridazine 300 mg q.h.s. and chloral hydrate 1000 mg q.h.s. she sleepwalked on 5 of the 6 nights recorded. On thioridazine 300 mg q.h.s. alone, she walked one night in four; on chloral hydrate 2000 mg alone, she did not walk during three nights of recording. All sleepwalking episodes occurred out of slow-wave sleep.

These results suggest that she sleepwalked only on psychotropic medications, particularly thioridazine. This case is similar to those reported by Huapaya (Am. J. Psychiatry 133: 1207, 1976) of somnambulism secondary to bedtime doses of methaqualone in two cases, chlorprothixene in another, and the combination of methaqualone and thioridazine in the fourth. In all cases sleepwalking could be abolished by either reducing or eliminating the bedtime dose. Two of the cases had childhood histories of sleepwalking, as did our own. As well, Flemenbaum (Am. J. Psychiatry 133:570-572, 1976) has reported cases of pavor nocturnus, another disorder associated with stage 4 sleep in patients receiving large bedtime doses of tricyclics or antipsychotics. The mechanism of this phenomenon, which may not be uncommon, is unknown. We are presently analyzing our patients' sleep EEG's.

IV. Major Findings: Normal Studies

A. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutes of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stages 3 and 4 ($p<0.05$, 2-way analysis of variance). Rapid eye movement (REM) and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimental night ($r=-.76$,

$p<0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2 and REM sleep resembled the waking state. Of interest was the finding that waking AER amplitude/stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhibition of sensory processing.

V. Major Findings: Animal Behavior

A. Deprivation of Rapid Eye Movement Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested. (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) Large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p<.05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

B. EEG Documentation of a Method for Sleep Depriving Rats

A variety of methods have been employed to produce total sleep deprivation in small animals. These include continuous handling, the use of treadmills or small platforms surrounded by water and administration of amphetamines or electric shocks. Unfortunately, these techniques are largely without support of EEG data. Most also have clear disadvantages. The use of small platforms surrounded by water is an outgrowth of a procedure for REM sleep deprivation; when used for purposes of total sleep deprivation it actually may cause a profound suppression of REM sleep with a smaller decrease in NREM sleep, which still occurs. Other approaches, such as continuous handling of the animals, require enormous amounts of labor. There is a need, then, for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. In this abstract we shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cylinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven animals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p<0.001$). The small amount of sleep which did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

We have presented a method of reducing total sleep to less than a tenth of normal for prolonged periods of time. Unlike the modified use of the platform surrounded by water, it does not totally suppress one sleep state (REM) while only partially suppressing another (NREM sleep). A much larger number of rats (12) can be studied at one time than in previous procedures. It is anticipated that this will be useful in observing the effects of sleep deprivation in studies dealing with memory, neurochemistry, and possible circulating sleep factors.

EEG ANALYSIS OF RATS IN SLEEP-DEPRIVATION APPARATUS FOR 24 HOURS*

	$\frac{\text{TOTAL SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{REM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{NREM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$
Control (n=7)	47.0 ± 3.8	7.4 ± 0.5	39.6 ± 3.7
Experimental Group (n=4)	3.8 ± 1.9	0.2 ± 0.0	3.6 ± 1.9
Significance**	$p<0.001$	$p<0.001$	$p<0.001$

* Values represent means ± SEM

** Two-tail t-test

VI. Studies of Human Memory:

A. Effects of Arecoline or Scopolamine Prior to Learning.

We determined the effects of arecoline and scopolamine on categorized serial learning in normal volunteers, all of whom were college students or recent college graduates. The task consisted of learning a fixed sequence of ten words belonging to a familiar category (such as vegetables, cities, or furniture). The list was repeated until all ten words were recalled in correct order on two successive trials. Prior to the testing session, subjects were pretreated with either methscopolamine or scopolamine. Both drugs block the peripheral cholinergic side effects of arecoline; since scopolamine crosses the blood-brain barrier, unlike methscopolamine, it also blocks the central effects of arecoline. Also, prior to testing, subjects received arecoline or saline subcutaneously.

The results indicated that arecoline (4 mg) significantly reduced the number of trials to criterion as compared with saline (mean 3.75 vs. 5.18 trials, $p<.01$). In contrast, the mean number of trials following scopolamine (0.5 mg) was 6.35 ($p<.01$, compared with 5.18 on methscopolamine-saline control). Administration of arecoline 6 mg, however, reversed the effects of scopolamine 0.5 mg (mean number of trials was 4.92, which was significant, $p<.01$, compared with scopolamine alone).

We also found that the major effects of arecoline and scopolamine were seen in naturally "poor" performers rather than "good" performers. The correlation between baseline, placebo performance and change on arecoline was $r=0.93$, $p<.001$, indicating that greater improvement was seen in those subjects who took the most trials to criterion on baseline; for scopolamine, the correlation was $r=0.5$, $p<.05$, indicating that the greatest decrement in performance was seen in those who took the most trials to criterion.

B. Effects of Arecoline Following Learning.

We showed that administration of arecoline immediately after learning facilitates later recall. Eight subjects (5 males, 3 females, mean age 24) were tested on two nonconsecutive days. Prior to the experiment, an intravenous needle was inserted in a forearm vein and attached to a ten-foot catheter extending out of the room so that drugs could be administered without the awareness of the subject. Following administration of methscopolamine (0.3 mg iv), subjects heard a list of words, nine groups of five words each. Three of the nine groups consisted of highly related (associated) words, three groups of weakly associated words, and three groups of randomly associated words. The groups were presented in order: first a highly related group, then a weakly associated group, then a randomly associated group, until all nine groups had been presented. After listening to each group of five words, subjects were asked to think of and say a word which defined or organized that word cluster. Immediately at the end of the session, subjects received a twenty minute infusion of either arecoline (2 mg in 20 ml of saline) or placebo (20 ml saline). Recall testing began 1 hour following the onset of the infusion. Subjects were tested first under free recall conditions and, then, under "cued" recall conditions (after having been presented with their own, self-generated cue word).

Compared with placebo infusion, arecoline significantly improved recall of the presented word; all three types of word sets--highly associated, weakly associated, and randomly associated--were better recalled ($p<.05$, $F=7.8$, $df=1,7$). This was true under both free and cued recall conditions. Interestingly, the arecoline effect appeared to be mediated by an increase in the number of clusters recalled ($p<.01$, $F=13.6$, $df=1,7$) rather than an increase in the number of words per cluster. While cued recall was better than free recall ($p<.001$, $t=29.6$, $df=1,7$), and highly associated word sets were better recalled than weakly or randomly associated words, the facilitory effect of arecoline did not interact with these factors.

C. Effects of Choline Chloride on Human Learning.

To study the effects of choline chloride in man, we administered an elixer containing 10 gm or placebo on separate days. Ninety minutes later, we began the session with two different tests: uncategorized serial learning and a selective reminding task.

Choline administration improved performance on both tests. On the uncategorized serial learning tasks, subjects learned to recite ten unrelated words in correct order; following each trial, the entire list was repeated by the experimenter. The number of trials to criterion was 5.7±0.7 after choline and 6.1±0.9 after placebo ($p<.05$, two-tailed t-test). On the selective reminding task, subjects learned a 12 word list of words, half of which were high image words (such as table) and half of which were low image words (such as virtue). Following each trial, the investigator reminded the subject of the words missed on the last trial, but did not repeat the words correctly remembered. As might be expected, high imagery words were learned in fewer trials on placebo than low imagery words, and choline did not alter the number of trials required for high imagery words. Choline administration, however, significantly reduced the number of trials required to learn low imagery words from 9.1±0.9 to 6.8±0.5 ($p<.01$).

The effects of choline were significantly correlated with performance on placebo, indicating a greater effect in "poor" performers than "good" performers. The change induced by choline was significantly correlated with performance on placebo on the uncategorized serial learning task ($r=0.59$, $p<.05$, $N=10$) and on low imagery words of the selective reminding task ($r=0.86$, $p<.01$, N-10).

The results of these studies, as well as those of many other investigations, strongly suggest that enhanced cholinergic activity facilitates certain aspects of cognition, memory, and learning. While these results raise the possibility of helping patients with learning disabilities with cholinergic agents, obviously much more work needs to be done. The effects of different pharmacological agents upon varying cognitive processes --short and long term memory, attention, structured and unstructured material--needs to be better specified. The nature of the various cognitive disabilities needs to be better defined, and the response of different abnormalities to different drugs also needs to be investigated. Issues of tolerance and long-term use have, also, not yet been addressed. In the absence of satisfactory methods (short of biopsy) for determining the diagnosis of Alzheimer's Disease, or specific abnormalities of central cholinergic systems, however, it may be important to establish individual differences in response to cholinergic therapies. Are there patients who consistently improve with cholinergic agents in repeated double-blind trials? Many problems remain, and no easy treatments can be readily anticipated. Nevertheless, we are now developing a series of clinical trials, in collaboration with Dr. Michael Ebert, of arecoline and lecithin in patients with dementia.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00098-05 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Study of Children and Grandchildren of Patients with Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
Other:	Donald H. McKnew, M.D.	Medical Officer	BP	NIMH
	Walter Sceery, J.D.	Social Worker	BP	NIMH
	Joel R. Hamovit, M.S.W.	Research Social Worker	BP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

XX (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, and to identify biological markers of a genetically-transmitted predisposition to affective disorders, and to study the interaction between genetic predisposition and environmental factors in children with affective disorders. Our present project is a refined replication of our pilot project reported last year. The major changes involve the inclusion of two control groups as well as a blind interviewer and rater.

Project Description:

Objectives: 1) to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness; 2) to identify biological markers of a genetically-transmitted predisposition to affective disorders; and 3) to study the interaction between genetic predisposition and environmental factors in children with affective disorders.

Methods Employed: We are studying three groups of children: 1) 50 children of parents hospitalized at NIMH with a primary affective illness; 2) 25 children of patients hospitalized at NIH because of a physical illness and screened for any affective disorder; and 3) 25 children of "normal" parents with no known physical or mental disorder. All children are seen twice, four months apart. After the second interview, blood is drawn on the 50 children in group 1 for determination of genetic markers as well as blood serotonin levels (in cooperation with Drs. Elliot Gershon and Dennis Murphy).

A social worker obtains at each visit a history from the parents and administers the Achenbach Scale for childhood psychopathology.

At each visit, the child has a psychiatric interview. The interview is conducted by a blind interviewer and rater, while another investigator observes through a one-way mirror. The interviews are structured, and follow a standard inventory of topics aimed at eliciting various types of psychopathology such as affective disorders, anxiety reaction in childhood psychosis, delinquency and minimal brain dysfunction. A portion of the interview is aimed at eliciting fantasy material by the use of free drawings, story telling, and free reporting of dreams, T.V. stories, and movies. Other methods used in child psychiatry such as the three wishes and the Despert fables are also employed. Following the interview, the children are rated on the CPRS scale of the ECDEU as well as on a global rating scale for affective disorders contructed for this purpose and used by the investigators for many years. All the above mentioned procedures pose no hazards for the children. In addition, after each visit, the children will be rated on the Weinberg scale.

Major Findings: In our pilot study of the 30 children, seven fell into the severe range and nine into the moderate range on a depression score devised by totaling the affective rating items in the various scales we used. None of the children were hypomanic or psychotic and there was no correlation between the depressive symptoms and the sex and age of the children. In our current project, we have seen so far 48 subjects as well as their parents. A preliminary analysis of our data on 48 children was done under the direction of Dr. John Bartko. (He employed safeguards to maintain the blind.) The results so far reveal significant differences of the presence of a depressive disorder between the probands and the control groups. A further analysis is still in progress.

Significance to Biomedical Research and the Program of the Institute: There is much interest in determining whether affective disorders can be rated to predict biochemical and other physiological measures which may be genetically determined. Also, there is a considerable interest presently in the nature and prognosis of affective disorders in children and their relationship with affective disorders in adults. It is to be hoped that our present study will help to clarify some of the issues mentioned above.

In addition to the potential significance of clarification of the problem of affective disorders in children, this study has the potential of developing preventive measures in children and perhaps (psychotherapeutic and pharmacological) measures to reduce and forestall, or reduce the frequency of, affective disorders in adults.

Proposed Course of Project: A) We will continue our ongoing project until all the 100 children have been seen. B)(1) We plan to continue to follow these patients twice a year for as long as possible. At each follow-up visit, there will be a history given by the parents, using the Achenbach Rating Scale, a psychiatric interveiw and rating of the child. (2) The parents of all the children will be seen in a conference and the findings will be discussed. When significant psychopathology is found, the family will be counselled about securing psychiatric help for the child.

Addendum: A sample of the above patients, including 20 children of manic-depressive parents, and 20 matched control children, is currently being re-evaluated using the above mentioned criteria plus the revised DSM-III diagnostic system.

Publications:

McKnew, D.H. Jr., Cytryn, L. Efron, A.M., Gershon, E.S., and Bunney, W.E. Jr.: Offspring of patients with affective disorders. Brit. J. Psychiatry 134: 148-152, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00100-04 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Aspects in Childhood and Adolescent Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	William E. Bunney, M.D.	Chief	BP	NIMH
	Monte S. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BP	NIMH
	Thomas M. Achenbach, Ph.D.	Research Psychologist	LDP	NIMH
	Christy L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS

COOPERATING UNITS (if any)
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LAB/BRANCH
Biological Psychiatry Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.60	1.60	3.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An inpatient program with selected overnight stays, for childhood and adolescent neuropsychiatric disorders is ongoing. The conditions currently under study are hyperactive children (HAC) and Gilles de la Tourette's Syndrome. Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, L-dopa, and tryptophan. Pharmacokinetic studies with clinical responses are ongoing. Central neurotransmitters and their metabolites are being studied in plasma and urine. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity response do not correlate with plasma amphetamine levels. These responses occur during the absorption phase as determined by serial plasma amphetamine following a single dose. Urinary 3-methoxy-4-hydroxy-phenyl glycol shows a time-related decrease during treatment with d-amphetamine; homovanillic acid is unchanged. Piribedil is safe, but clinically ineffective in HAC.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this program are broad. In the most basic sense, the objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with especial reference to maturational changes. Compared to the neurobiology known in adult neuropsychiatry, much less is known regarding the neuropsychiatric disorders of children. An integration is a pressing need.

Several principles are important to keep in mind in the study of children versus adults: 1) morphological brain maturation is age related, i.e., the reticular activating system, of considerable importance in psychobiology, is not fully myelinated until ages 10 to 20; 2) children may have more rapid and dramatic pharmacological responses, both therapeutic and toxic; 3) no clear history of pre-adolescent "addiction" to compounds, i.e., amphetamine, which can effect unequivocal addiction in adolescents; 4) most behavioral symptoms in children that show pharmacological responsivity have more of an apparent organic contribution than those of adults, i.e., motor hyperactivity, enuresis, poor attention span in children versus complex emotional and cognitive aberrations in adults; 4) clinical symptomatology is more variable and fluctuating in children than usually seen in adults, i.e., rating scales for endogenous depression in adults are more easily constructed to measure a "constellation" than is the case for childhood depression; well formed delusions and hallucinations are not so common in young psychotic children as in adults.

In addition, a number of studies, pharmacological and otherwise, are being undertaken to elucidate cerebral metabolism in specific disorders and possible protein defects in those disorders.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, has been established on the 2 West Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a more closely defined population of HAC. Gilles de la Tourette's syndrome is also being studied. Children with autism and various metabolic defects can and may also be studied.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). A parents' questionnaire developed by Achenbach which divides children into nosological groupings by factor analysis is being scored blindly on all admissions and will be compared to those diagnoses ascertained in the usual clinical way and by research rating instruments specific for a disorder (as hyperactivity).

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for methylphenidate and amphetamine. These data are studied in conjunction with motor activity, behavior, and cognition. Piribedil, a specific dopamine (DA) agonist, has been given to HAC, and clozapine, a neuroleptic, has been given in Gilles de la Tourette's syndrome. Both L-dopa, a precursor of DA, and tryptophan (TA), a precursor of serotonin (5HT), are being administered to HAC as clinical trials.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

Amphetamine is measured by a radioimmunoassay (RIA) and gas chromatograph-mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study homovanillic acid (HVA), 3-methoxy-4-hydroxyphenyl glycol (MHPG) and 5-hydroxyindoleacetic acid (5HIAA). Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma norepinephrine (NE) changes as they relate to plasma amphetamine levels have also been studied. Currently, plasma changes in MHPG and HVA post amphetamine administration are being measured. Plasma TP and 5HIAA will also be measured in the 5HT study. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average Evoked Response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes are also studied in relation to amphetamine, piribedil, and TP effects. Paired associate learning has also been assessed in different drug conditions.

Major Findings: Significant correlations exist between community teachers' and NIH teachers' CTRS ratings of behavior.

Serial plasma pharmacokinetic data indicate that children reach a peak serum level of amphetamine within 3-4 hours (h) of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data is highly replicable. Clinical responses have also been studied after use of sustained release capsules. This preparation produces a slower rate of absorption and a more plateau-like, longer lasting peak level, but does give a prolonged clinical response. Socially appropriate behavioral change, increased attention span, and concentration ability is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-amphetamine during its absorption phase. This data indicates that the clinical changes may be related to depletion of catecholamine stores, to replacement by a "false" neurotransmitter metabolite of amphetamine, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect similar clinical responses, but less so. Piribedil is safe, but clinically ineffective for HAC.

Study of urinary metabolites indicate that day and night excretion of MHPG and HVA are not significantly different; however, d-amphetamine after eight days is associated with significantly lower MHPG levels and the data indicates that behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged.

HAC are not significantly different from normal controls with regard to plasma NE and dopamine-β-hydroxylase (DBH) but do have significantly more neurological soft signs by PANESS examination. Plasma NE is significantly correlated with anxiety as well as being significantly elevated at 2-3 h following a single oral dose of d-amphetamine.

Studies evaluating CPT are now underway; amphetamine is effective and piribedil is minimally so. AER studies are preliminary. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full scale IQ (WISC-R) and a greater number of errors on the Bender. Data from psycholinguistic evaluations are being prepared for publication.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological, and psychobiological questions to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, Gilles de la Tourette's syndrome, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and amphetamine give positive responses in 80% of well diagnosed HAC, and likewise, haldol gives a high frequency of positive response in Gilles de la Tourette's syndrome, the pharmacokinetics and metabolism of these drugs have not been closely studied in children. The relationships between such basic pharmacological knowledge and clinical effects have been understudied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: We plan to conduct this program over an indefinite period.

Publications:

Caine, E.C., Margolin, D., Brown, G.L., and Ebert, M.H.: Gilles de la Tourette's syndrome, tardive dyskinesia, and psychosis in an adolescent. Am. J. Psychiatry 135: 241-243, 1978.

Brown, G.L., Ebert, M.H., and Hunt, R.D.: Plasma d-amphetamine absorption and elimination in hyperactive children. Psychopharmacol. Bull. 14(3): 33-35, 1978.

Rapoport, J.L., Mikkelsen, E.J., Ebert, M.H., Brown, G.L., Weise, V.K., and Kopin, I.J.: Urinary catecholamines and amphetamine excretion in hyperactive and normal boys. J. Nerv. Ment. Dis. 166(10): 731-737, 1978.

Achenbach, T.M., and Edelbrock, C.S.: The classification of child psychopathology: A review and analysis of empirical efforts. Psycopharmacol. Bull. 85: 1275-1301, 1978.

Brown, G.L, Hunt, R.D., Ebert, M.H., Bunney, W.E. Jr., and Kopin, I.J.: Plasma levels of d-amphetamine in hyperactive children: Serial behavior and motor responses. Psychopharmacology 62: 133-140, 1979.

Brown, G.L., Ebert, M.H., Mikkelsen, E.M., and Hunt, R.D.: Clinical pharmacology of d-amphetamine in hyperactive children. In Gottschalk, L.A. (Ed.): Pharmacokinetics and Clinical Response. New York, Spectrum Publications, 1979, (In Press).

Powers, K.H., and Ebert, M.H.: Comparison of RIA and GC-MS assays for d-amphetamine. Biomed. Mass Spectrometry, 1979 (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00101-03 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Childhood Enuresis: Clinical Response to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .65	PROFESSIONAL: .40	OTHER: .25

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies investigated severe enuresis in males, aged 6-12, using electroencephalographic measures during treatment with placebo or active drugs thought to benefit the condition. Half of the subjects are selected to have behavioral disturbance, while half have enuresis as a single symptom. The state of sleep during which enuretic events occurred were recorded as well as other sleep parameters. At the present time, 40 children have been studied.

Project Description:

Objectives: The purpose of this first study was to elucidate the mechanism by which imipramine is clinically effective in enuresis. A second purpose was to compare the sleep patterns of behaviorally disturbed and non-behaviorally disturbed children at baseline and during treatment with imipramine, an antidepressant in general use for a variety of childhood disorders. Enuresis has a strong association with childhood psychiatric disorder although it also occurs as an isolated symptom. It is hoped that through the study of the mechanism by which imipramine acts, and of the sleep patterns of these patients, the underlying pathophysiology of the disorder will be better understood.

Methods Employed: Patients were initially selected by telephone screening on the basis of the severity of the enuresis, and whether the child could be clearly identified as belonging in one of the study groups, i.e., with associated behavioral disturbance, or with enuresis as an isolated symptom. The Peterson Quay behavior checklist was used for this purpose. On admission to ward 2-West, a medical, neurological and psychiatric examination was carried out. Three nights of baseline sleep recordings were made. Utilizing a structured psychiatric interview for use with children, a diagnostic evaluation is made during the baseline period. Following three nights of baseline sleep recording, the children are given three sequential outpatient trials of medication. The first period was a single-blind placebo trial, followed by a double-blind crossover trial of methscopolamine (6 mgm hs) or imipramine (75 mgm hs). At the end of each 2-week trial period, sleep recordings were repeated and blood samples obtained for blood level of imipramine and desmethylimipramine.

Major Findings: Forty-two subjects were studied to date; there have been two dropouts from the study. The most striking finding from baseline evaluation was that enuretic events occur during all sleep stages and not, as had been previously reported, primarily during slow wave sleep. A) No subject responded to placebo. B) There was a significant difference between the efficacy of the two active drugs; imipramine produced a significant decrease in enuretic episodes while methscopolamine did not. These findings indicate that imipramine action in enuresis is central rather than peripheral, as methscopolamine has only peripheral anticholinergic activity. C) Plasma tricyclic concentration showed some correlation with clinical antienuretic response. However, true pharmacological tolerance occurred in approximately one-third of the sample.

Significance to Mental Health Research: As enuresis is strongly associated with other psychiatric disturbance of childhood, a study of the pathophysiology of the condition may shed light on more general physiological disturbance. The comparison between disturbed and non-disturbed enuretic children may differentiate two separate conditions, an "arousal" disorder and a disorder related to "lighter" sleep stages more common in behaviorally disturbed children. Methscopolamine has peripheral anticholinergic action but does not cross the blood brain barrier. The failure of methscopolamine to be effective in enuresis supports a central action of imipramine although other peripheral effects (such as noradrenergic potentiation) cannot be ruled out. The data from the first study with 14 children appears sufficiently clear. Therefore, the project has been amended and a new study (Project No. 76-M-384-A) will be conducted to further elucidate the mechanism of action of imipramine in this condition (see below).

Proposed Course of Project: As both imipramine and desmethylimipramine are equally effective in enuresis, a trial of Iprindol which does not block reuptake of catecholamines would be of interest to evaluate whether the antienuretic effect is necessarily tied to the effect on catecholamine metabolism.

Publications:

Mikkelsen, E., Rapoport, J., Mendelson, W., Gruneau, C., and Gillin, C.: Enuresis I: Sleep stages and clinical status. Arch. Gen. Psychiatry (In Press).

Rapoport, J., Zavadil, A., Mikkelsen, E., Nee, L., Mendelson, W., and Gillin, C.: Enuresis II: Plasma tricyclic concentration and clinical antienuretic response. Arch. Gen. Psychiatry (In Press).

Potter, W., Zavadil, A., Mikkelsen, E., Rapoport, J., and Goodwin, F.: Hydroxy-metabolites of tricyclic antidepressants: Relationship to clinical efficacy and side effects. Psychopharmacology Bulletin (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00102-02 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Lithium Response in Children of Lithium Responding Manic Depressive Parents
Formerly: Childhood Enuresis: Clinical Responses to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

XX (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of lithium in emotionally disturbed children having at least one manic-depressive lithium responsive parent will be examined. Thirty children, ages 5-15, of both sexes, having psychiatric symptoms that are in some way incapacitating and are of major concern to the child, parent, or teacher will be selected. Children will be given lithium and placebo, in a double-blind, crossover design for 16 weeks. Behavior ratings will include: parent's and teacher's assessment, psychophysiological measures, child interview, and cognitive testing. The lithium administration will be monitored by an "unblind" co-investigator.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To test the hypothesis that some of the offspring of lithium responding parents will prove clinically responsive to lithium. 2) To test the hypothesis that children with an affective and/or cyclic component to their psychopathology will be better responders. 3) To see if the response to lithium may possibly be a genetic marker for manic-depressive illness. 4) To identify the children at risk with early prodromal disorders who respond to lithium, prior to the outbreak of manic-depressive illness.

Methods Employed: The 30 patients will be admitted to the study upon recruitment from the NIMH Outpatient Department (OPD) as well as from the local psychiatric community. The fact that the parents are indeed lithium-responding manic-depressives will be independently verified on the basis of direct examination and medical records of parents.

The patients will be selected on the basis of having sufficient psychopathology for it to be incapacitating and to be of concern to their parents and/or the school authorities. Any children with significant brain damage and/or an IQ below 80 will be excluded from the study. The patients will be ages 5-15, of both sexes, and with no regard for their socio-economic status. These patients will be admitted to the Outpatient Clinic for the duration of the study (16 weeks). Patients of special interest may be retained in the OPD for follow-up treatment and study. Other patients will be returned to their previous source of care or referred to local psychiatrists, as needed.

Their work-up records will be handled as described in the Handbook for Staff Physicians.

Admission Work-Up:

Parents: A structured history will be obtained. In addition, the parents will be requested to fill out the following questionnaires: 1) Deviant Behavior Inventory (DBI); 2) the Achenbach Scale; and 3) the Conners' Parent Questionnaire.

Patients: A diagnostic evaluation will be obtained including a structured interview and a routine physical exam. On the basis of all available information, a clinical diagnosis based on DSM-III will be made. Any affective component will be evaluated using the Weinberg criteria for depression and mania. The following rating scales will be filled out: 1) Children's Psychiatric Rating Scale (CPR of the ECDEU); 2) Periodicity Rating; and 3) Children's Affective Rating Scale (CARS). In addition, a global rating on a 7-point scale will be administered.

Blood will be drawn for BUN and T_3, T_4 to rule out renal or thyroid pathology. These tests will be repeated at the end of the study.

The physiological test, Average Evoked Response (AER), will be administered. The AER may help to predict lithium responders who have a greater visual AER, termed augmenting.

Teachers will fill out the full Conners' Teacher Rating Scale (CTRS).

The thirty patients will be randomly assigned to 2 groups (I and II). Group I will be treated as follows: 4 weeks placebo, 4 weeks lithium, 4 weeks placebo, 4 weeks lithium. The procedure will be reversed in Group II.

Nature of Procedure: Lithium will be administered in blind fashion except that a case "coordinator" will be unblind. The first lithium administration will begin with the mean child dose for that age and will be adjusted until a blood level between .8 and 1.2 mg/liter is obtained. The case coordinator will adjust the number of tablets for a matched placebo case in parallel with adjustment for active drug.

Side effects will be monitored by using a lithium side effect checklist (see Appendix I). The second period of lithium administration will begin with that child's effective dose.

1) The patients will be seen on a weekly basis for evaluation and administration of medication. At each visit the following procedures will be carried out: A brief structured interview of 15-30 minutes with a special focus on the target symptoms. The interview will be rated on the target items of the CPRS and the CARS. Lithium levels will be obtained both in serum and saliva. The Side Effect Checklist will be filled out by the "unblind coordinator" who will also adjust the dosage at that time. The global rating scale will be repeated.

The AER will be repeated on a schedule regulated by the unblind lithium administration.

Major Findings: None to date.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00151-03 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavior and Electrophysiological Response of Normal and Hyperactive Children and Normal Adults to a Single Dose of Dextroamphetamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Irwin J. Kopin, M.D.	Chief	LCS	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study was to study the effects of a single dose of dextroamphetamine on normal children and adult males. The initial protocol established the fact that normal children show a response to amphetamine qualitatively similar to that of hyperactive children. An amended protocol investigated the response of normal adults to stimulant using the identical tasks used in hyperactivity research.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To determine the nature of response of normal adults to dextroamphetamine in the test situation usually employed in hyperactive child research. 2) To correlate ratings of childhood and adult hyperactivity with response to stimulant within the normal adult sample.

Methods Employed: During the period covered 30 normal adults were examined at baseline and during a double-blind crossover administration of either placebo or dextroamphetamine. One group received 0.5 mg/kg and a second group received .25 mg/kg. All subjects were admitted to ward 2-West and received a test battery including reaction time and GSR, Continuous Performance Test, Paired Associate Learning and Porteus Maze Test. Motor activity was recorded during the test session.

Major Findings: Fourteen normal male children, fifteen hyperactive children, and 30 college-age males were tested. Most subjects moved less during testing while on drug. There was an increased vigilance, decreased reaction time and improved learning of verbal material. The changes for adults were similar to those seen with hyperactive children for the most part. The absolute dose given the first adult group was rather large (35 mg/subject). A second group tested on a lower per weight dose had a more similar response to that seen in children.

Significance to Biomedical Research and Program of the Institute: The diagnostic significance of a beneficial response to stimulant drugs is of major interest both to child psychiatry and general psychiatry. If hyperactive children respond to stimulants in an identical fashion to normal children, then it is not necessary to postulate hypoarousal of dopamine deficiency as a necessary basis for clinical response. Moreover, some animal models of hyperactivity are now seen as erroneous as these employ animals who become excited following stimulants unless chemical or anatomical lesions are made. The current protocol suggests that this is an invalid model for clinical hyperactivity.

The adult subjects were all judged to be currently free from psychiatric illness; however, they varied widely with respect to retrospective reports of childhood behavior. The relationship between childhood history and adult behavioral response to stimulant is being examined and is relevant to the growing interest in the use of stimulant drug treatment in "Adult Minimal Brain Dysfunction."

Proposed Course: The project will be completed during the period covered in this report.

Publications:

Rapoport, J., Buchsbaum, M., Weingartner, H., Zahn, T., and Ludlow, C.: Dextroamphetamine: Behavioral and cognitive effects in normal prepubertal boys. Science 199:560-563, 1978.

Ludlow, D., Rapoport, J., Cardano, C., and Mikkelsen, E.: The differential effects of dextroamphetamine on the language and communications skills of hyperactive and normal children. In Knights, R., and Bakke, H. (Eds.): Learning Disabilities -- 2nd NATO Conference. Baltimore, University Park Press (In Press).

Zahn, T., and Rapoport, J.: Autonomic effects of stimulant drugs in normal and hyperactive children. J. Abn. Child Psychol (In Press).

Rapoport, J., Weingartner, H., Buchsbaum, M., Ludlow, C., Mikkelsen, E., and Langer, D.: Dextroamphetamine: Cognitive and behavioral effects of normal and hyperactive children and normal adults. Arch. Gen. Psychiatry (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00152-02 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Sinemet on Behavior in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Gerald L. Brown, M.D.	Medical Officer	BP	NIMH
	Michael H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH
	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	C. Raymond Lake, M.D.	Medical Officer	LCS	NIMH
	Irwin J. Kopin, M.D.	Chief	LCS	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A double-blind crossover study is planned to evaluate the clinical response of approximately 15 hyperactive boys to Sinemet (maximum dose, 60-600 mg). The behavioral and cognitive responses will be correlated with changes in plasma norepinephrine and measures of dopamine turnover.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive children usually respond symptomatically to stimulant medications which are known to increase the concentration of monoamines at the receptor sites in the brain. As L-dopa is known to increase the availability of dopamine in the brain, if a deficiency of dopamine were responsible for at least some cases of hyperkinesis, then L-dopa should be expected to have some therapeutic value.

Methods Employed: The recent practice of combining L-dopa and Carbidopa, a peripheral dopa decarboxylase inhibitor, lowers the necessary dosage and thereby decreases side effects, making this treatment more feasible for children. Hyperactive boys, ages 6-12, exhibiting restlessness and impulsivity both at school and during an inpatient evaluation period will be selected for the protocol. Evaluation will include medical, neurological, and psychiatric examination as well as psychological testing.

Children will be started on a gradually increasing dosage of Sinemet or placebo; medication will be increased over the first two weeks to the maximum dose (60-600 mg Sinemet or 6 tablets of placebo), which will be given during the third week as tolerated.

During the third week of placebo or Sinemet treatment, plasma norepinephrine and HVA turnover will be assessed. Clinical response for the group will be compared for drug and placebo periods and individual responses will be related to changes, if any, in the catecholamine measures.

Major Findings: The project began in June 1978. Six children have received the drug. Preliminary impressions are that Sinemet is not useful in control of hyperactivity.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism or schizophrenia, studies on treatment and underlying pathophysiology of hyperactive children have wide application throughout the field of mental health research. Alternate treatments to stimulants are practically useful, as approximately 15% of hyperactive children do not respond favorably to stimulants.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00153-02 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Treatment of Obsessional Children and Adolescents with Chlorimipramine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Robert N. Elkins, M.D.	Clinical Associate	BP	NIMH
	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
	Dennis L. Murphy, M.D.	Chief	CN	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .10	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessional disorder of childhood is a rare disorder about which little is known. The purpose of this protocol is to collect basic data on family history of mental illness, sleep measures and neuropsychological testing on a selected group of children and adolescents with obsessional disorder. A drug trial of chlorimipramine, desmethylimipramine, and placebo will be carried out to evaluate the effect of antidepressant and the specificity of chlorimipramine for this disorder.

Project Description:

Objectives: To evaluate the clinical response of Obsessive-Compulsive disorder of childhood to chlorimipramine and to relate this response to changes in ratings of depression. Clinical response to chlorimipramine will be related to platelet serotonin at baseline.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory will be used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 16-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of drug as well as platelet serotonin will be assayed for each drug phase.

Major Findings: Seven children have entered the protocol: six males aged 13-15, and one 13-year-old female. The first patient improved gradually throughout his hospital course and did not have a different course during chlorimipramine treatment. Depression ratings changed in parallel with obsessive symptoms. One patient showed dramatic improvement on chlorimipramine and is being maintained on the drug. The others have either not completed the protocol or showed equivocal response.

Preliminary analysis of family pedigree data indicates high incidence of depression among first degree relatives. Sleep recordings on subject No. 1 showed marked decrease in REM latency at baseline, which increased over time with clinical improvement. These findings indicate a possible biologic link between depression and obsessive-compulsive disorder in childhood. Speech and language testing suggests mild dysphasia suggestive of a dysfunction of frontal and/or temporal lobes.

Proposed Course of Project: A total of 20 patients will be studied. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to the active drugs.

Publications:

Elkins, R., Rapoport, J., and Lipsky, A.: Obsessive-Compulsive disorder of children and adolescents. J. Am. Acad. Child Psychiatry (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00154-01 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

State Dependent Learning in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	Herbert Weingartner, Ph.D.	Acting Chief	LP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.10	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

State dependent learning has primarily been demonstrated in adults receiving substances which impair learning. However, two studies have raised the possibility that hyperactive children who often learn material on stimulant drug may manifest state dependency as measured by decreased transfer of learning to the off-drug state.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive boys who also are at least one year behind age/IQ norms in academic achievement will be tested on and off dextroamphetamine (0.5 mg/kg) to see whether verbal material is best retained in the same or different state from which it is learned.

Methods Employed: Twelve hyperactive boys will be tested in four paired learning and retention sessions. Learning and recall will be measured during same (drug-drug or placebo-placebo) sessions and different (placebo-drug or drug-placebo) sessions. All sessions will be carried out using a double-blind, random assignment, counterbalanced order design. Mood scale ratings will also be completed for each child for each test session.

Major Findings: A total of nine children have been studied to date. The code has not been broken and so the effect of drug on learning and the effect of state change on recall is not known.

Proposed Course of Project: The study will be completed by October 1979. If state dependency is not demonstrated, then the study will terminate. If state dependency is found, it is probable that we will evaluate state dependency in children receiving chronic amphetamine treatment, as this is more applicable to clinical use.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00161-01 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cognitive Effects of Dietary Substances in Normal and Learning Disabled Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	Robert N. Elkins, M.D.	Clinical Associate	BP	NIMH
	J. Christian Gillin, M.D.	Research Psychiatrist	BP	NIMH
	Herbert Weingartner, Ph.D.	Acting Chief	LP	NIMH
	N. Sitaram, M.D.	Staff Psychiatrist	BP	NIMH
	Michael H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH;
Section on Experimental Therapeutics, LCS, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .30	PROFESSIONAL: .20	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both caffeine and lecithin, substances within the diet of normal children, have been shown to affect memory and vigilance of normal adults. The effects of these substances will be studied on normal children. If results look promising, the effects of lecithin on cognition of learning disabled children will be investigated.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To evaluate the effects of caffeine (4 and 10 mg/kg) in single doses and lecithin (60 gm/day for up to 5 days) on memory and vigilance of normal children.

Methods Employed: Children will be paid as normal volunteers for participating in this study. Children must be free from psychiatric disorder or cognitive disability.

Double-blind single dose trials of caffeine or lecithin will be carried out, using cognitive test response as the dependent measures. Side effects will also be evaluated.

Major Findings: These studies will start during the summer and fall of 1979.

Proposed Course of Project: Project has not yet started.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00034-09 BP

PERIOD COVERED October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychological and Physiological Correlates of the Average Evoked Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clin. Psychophysiology	BP NIMH
OTHER:	R. Coppola, D.Sc.	Electrical Engineer Sect. on Clinical Psychophysiology	BP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Clinical Psychophysiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evoked potentials at varying light intensities were examined in paranoid and nonparanoid groups of schizophrenics and in rapidly cycling bipolar affective disorder patients. Early components at 100-140 milliseconds differentiated paranoid from nonparanoids but did not change with mood cycles; late components did vary with affective mood.

PHS-6040
(Rev. 10-76)

Project Description:

Evoked potential research has been given emphasis by the unit because evoked potentials show high heritability and sensitivity to attentional as well as neuropharmacological manipulation -- coupled with the possibility of cortical or subcortical localization, these features make it an attractive technique for biological research in psychiatry.

Electrophysiological measures of perceptual processing suggest increasing inhibition of input as individuals mature through the 15-50 age range. The diminished EP size and the change in amplitude/intensity slope functions toward "reducing" especially support this concept. Neurophysiological studies of the visual reducing phenomenon in cats suggested that reducing does not depend on peripheral visual mechanisms or differences in thalamic responsiveness but that the phenomenon occurs cortically following the interaction of ascending visual stimuli with brainstem arousal mechanisms. Interestingly, the nonspecific thalamic radiations, reticular formation, intracortical association fibers and the supralimbic cortex are among the latest to show myelination.

From over 100 normal adult volunteers who have participated in one or more experiments on psychophysical and pharmacological correlates of analgesia, we selected subjects under 30 (28 men and 17 women) and over 40 (9 men and 19 women). All subjects had participated in an identical first session which consisted of two psychophysical pain rating procedures and somatosensory EP recordings. No drug or placebo was given. Electrical stimulation was provided by a Tursky concentric electrode (1974) connected to an on-line computer controlled constant current stimulator and placed on the dorsal surface of the left forearm.

As with the visual EPs, less increase in EP amplitude with increasing intensity was seen for the P100 component for older subjects. This was confirmed for both amplitude measurements (two-way ANOVA, p 0.05 age by intensity interaction, quadratic effects F=6.08 and 7.88, 1 and 69 d.f. for Cz and C4 leads) and for the area integration technique (F=5.37, p 0.05 for the Cz lead). Simple age diminution was found for the P200 component at Cz for the amplitude measure (two-way ANOVA, age effect, F=5.81, 1, 69 d.f., p 0.05) as well as for the area integration measure (F=8.03, p 0.01). Latency differences for the various EP components were not statistically confirmed.

Pain judgments paralleled the EP findings of diminished pain sensitivity with age although the results were not as strong. Females tended to show stronger increases in pain tolerance with age than males.

Age comparisons with bipolar and schizophrenic patients are currently under way to evaluate differences in age-related changes in the major psychoses.

Visual evoked potentials (VEP) are clearly nonlinear in nature since in many cases a sine wave modulated light (SML) stimulus produces its major EEG response at twice the frequency of stimulation. It has previously been shown that nonlinear analysis may be applied to the human VEP by collecting VEPs to white noise modulated light. Having developed a method to identify the system kernels, the prediction of the system response to any temporally modulated light signal is possible. In this case, the linear and nonlinear kernels give the fundamental and harmonic response. The frequency characteristics computed in this way agree favorably with the spectrum analysis of SML data. The complete predicted response also agrees favorably with the actual steady state VEP (SSEP). Using this method of analysis the variation in wave shape of the SSEP is seen to be a function of the differential gain and latency of the linear and nonlinear parts. The kernels may also be used to predict the transient visual response (averaged VEP). The different responses seen to increasing intensities of stimulation seem to be related to the different gains of the linear and nonlinear components of the response revealed by this type of analysis. Comparison to actual responses in terms of wave shape suggests that parallel linear and nonlinear pathways might exist in the visual system and that a combination of the potentials due to each pathway may account for the variation in frequency and intensity response seen in VEPs.

An autoregressive filter whose frequency transfer characteristic can be easily controlled was developed as a method for isolating and removing very slow activity from EEG records prior to spectrum analysis. This development will aid in determining whether the low frequencies often seen in EEG records is artifactual in nature or actually represents some physiological process.

Cortical potentials to visual stimulation by gratings of various spatial frequencies have been obtained from alert rhesus monkeys in order to gain some insight into the processing of visual pattern information. Findings are similar to human responses to checkerboard stimulation. Enhanced capability for visual pattern stimulation is being developed. Hopefully this will lead to better methods for assessment of human psychophysiology.

Publications:

Coppola, R.: Isolating low frequency components in EEG spectrum analysis. Electroenceph. clin. Neurophysiol., 46:224-226, 1979.

Coppola, R.: A system transfer function for visual evoked potentials. In D. Lehmann and E. Callaway (Eds.): Human Evoked Potentials: Applications and Problems, New York, Plenum, 1979.

Buchsbaum, M.S. and Coppola, R.: Signal-to-noise ratio and response variability in affective disorders and schizophrenia. In Begleiter, H. (Ed.): Evoked Brain Potentials and Behavior. New York, Plenum, 1979, pp. 447-465.

Coppola, R.: Computer applications in nonlinear modeling of EEG. Brain Theory Newsletter, 3: 111-118, 1978.

Coppola, R.: Functional power series analysis of visual evoked EEG suggests parallel linear and nonlinear pathways. Neurosci. Abstracts, 4:152, 1978.

Buchsbaum, M.S.: Electrical activity of the brain: Age changes and personality differences in: Proceedings of Council Seminars, Duke University, Center for the Study of Aging and Human Development, Duke University Medical School, Durham, N.C., (In Press).

Nurnberger, J.I., Gershon, E.S., Murphy, D.L., Buchsbaum, M.S., Goodwin, F.K., Post, R.M., Lake, C.R., Guroff, J.J., and McGinniss, M.H.: Biological and clinical predictors of lithium response in depression. In Gershon, S., Kline, N.S., and Schou, M. (Eds.): International Lithium Conference: Controversies and Unresolved Issues. New Jersey, Excerpta Medica, ICS Series, (In Press).

Coppola, R. and Nakamura, R.K.: Electrocortical responses to visual spatial frequency in behaving monkeys. Neurosci. Abst., (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00035-07 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biochemical and Psychopharmacological Correlates of the Average Evoked Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.S. Buchsbaum, M.D.	Chief, Sect. on Clinical Psychophysiology	BP	NIMH
OTHER:	F.K. Goodwin, M.D.	Chief,	CP	NIMH
	D.L. Murphy, M.D.	Chief,	CN	NIMH
	T.P. Zahn, Ph.D.	Research Psychologist	LPP	NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Laboratory of Psychology and Psychopathology; DCBR, NIMH. Dept. of Clin. Psych., Univ. of Md.; Dept. of Psychiatry, (continued)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 0.6	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A population of individuals potentially at risk for psychiatric disorders was identified by screening 375 college student volunteers for low platelet monoamine oxidase (MAO) activity levels. The lower and upper 10 percent in MAO activity were interviewed and average evoked response, personality, perceptual and family history data obtained. Low MAO probands reported more frequent psychiatric or psychological counseling and problems with the law. Families of low MAO probands had an eight-fold increase in suicide incidence over those of high MAO probands. Both in these low MAO probands and in NIH psychiatric inpatients the combination of low MAO and average evoked potential augmenting was associated with increased frequency of suicide or suicide attempts. This suggests that reduced MAO levels, reported previously in patients with affective disorders and chronic schizophrenia, may predict a vulnerability to psychiatric disorder.

Cooperating Units (Continued)

University of Ky.; Dept. of Psychiatry, Brown University, Providence, R. I.; Dept. of Psychiatry, University of Minnesota, Minneapolis, Minnesota.

Project Description:

We previously found that the combined multivariate use of platelet MAO level and AER allowed us to successfully separate the four groups; normal, schizophrenic, bipolar, and unipolar affective illness, with 72% accuracy. However, since hospitalization stress, sensory isolation, dietary changes, etc., might affect both MAO and AER, we planned to locate normal individuals with extreme values of MAO and to study them with AER measures. This then represented a kind of biochemical "high-risk" study for the major psychoses.

The "high-risk" approach to the study of psychiatric disorders attempts to transcend the cause-effect dilemma by focusing on individuals believed vulnerable to such disorders before they become manifest. To select a pool of vulnerable individuals, nearly all high-risk studies have relied on genetic models and have examined the offspring or relatives of a clearly defined patient sample. Biochemical studies of psychiatric disorders, on the other hand, have usually begun with patients in "known" diagnostic groups and searched for abnormalities. The present series of studies integrates the usual biochemical paradigm with the high-risk one to produce a new biochemically-at-risk strategy. Here, the "proband" is established by a biological measure, not a clinical one. The use of this strategy reduces the heterogeneity of the sample; eliminates the hospitalization, labeling and treatment factors that confound other biochemical studies; and rigorously tests the power of the biological factor under investigation.

Results from two independent studies indicate that nonhospitalized individuals with psychopathology can be identified with a combination of a biochemical and a neurophysiological measure. In the first study, college students are selected for high or low platelet monoamine oxidase (MAO) activity and then classified according to an average evoked potential measure of augmenting/reducing (AR). In the second study, a new group of college students are selected for elevated MMPI scores and then classified according to the MAO and the AR measures as in the first study. Four biological groups, low MAO/reducing, are compared with respect to the prevalence of psychopathology. On the basis of either MMPI interpretations or the Research Diagnostic Criteria, more psychopathology is found among low MAO/augmenters and high MAO/reducers than among the other two groups. These results are interpreted as showing that specific biological patterns may be related to a general psychiatric vulnerability.

Chronic alcoholic patients were also studied, in collaboration with Dr. A. Ludwig, Chairman, Dept. of Psychiatry, University of Kentucky. It is hypothesized that alcoholics possess a perceptual neurophysiological sensitivity which renders them vulnerable to the effects of information or stimulus overload; the resort to alcohol, therefore, may represent an adaptive attempt to restore physiologic homeostasis through the pharmacologic "dampening" properties of this drug. Twelve alcoholic subjects were assigned to three different levels of audio-visual sensory stimulation (no, medium, high) and three different doses of alcohol (placebo, low, high) according to a balanced incomplete block design.

Data on visual evoked potentials are currently being analyzed and will be reported next year.

Visual evoked potentials were recorded promptly at the end of each period of sensory stimulation. The EP data are consistent with the hypothesis of a special neurophysiological vulnerability to sensory stimulation in alcoholics, with resort to alcohol apparently enhancing inhibitory sensory processes. Normal individuals showed a shift toward EP reducing following sensory stimulation, consistent with our earlier findings with noxious auditory stimulation and for somatosensory EPs with audioanalgesia. In contrast, alcoholics augmented more following sensory stimulation. The effect of alcohol was also opposite, controls showing augmenting and alcoholics showing reducing with a linear dose-response curve. The pattern of greater EP reducing following increased sensory input sessions seen in normals while sober was matched best by the data for high dose alcohol in the alcoholics. This finding of diminished sensory inhibition in alcoholics especially following sensory stimulation would predict their reported positive reaction to experimental sensory deprivation conditions. The resort to alcohol thus might represent an adaptive attempt to restore physiological sensory homeostasis.

Publications:

Buchsbaum, M.S. and Ludwig, A.M.: Effects of sensory input and alcohol administration on visual evoked potentials in normal subjects and alcoholics. In H. Begleiter, (Ed.): Biological Effects of Alcohol. New York, Plenum, (In Press).

Haier, R.J., Buchsbaum, M.S., Murphy, D.L., Gottesman, I.I., and Coursey, R.D.: Psychiatric vulnerability, monoamine oxidase and the average evoked potential. Arch. Gen. Psychiat., (In Press).

Buchsbaum, M.S., Murphy, D.L., Coursey, R.D., Lake, C.R., and Zeigler, M.G.: Platelet monoamine oxidase, plasma dopamine-beta-hydroxylase and attention in a "biochemical high risk" sample. J. Psychiatric Research (In press).

Coursey, R.D., Buchsbaum, M.S. and Murphy, D.L.: Platelet MAO activity and evoked potentials in the identification of subjects biologically at risk for psychiatric disorders. British J. Psychiatry, (In press).

Lipper, S., Murphy, D.L., Slater, S. and Buchsbaum, M.S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00036-05 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Individual Differences in Sleep and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clin. Psychophysiology	BP	NIMH
OTHER:	J. C. Gillin, M.D.	Research Psychiatrist	BP	NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP	NIMH
	W. B. Mendelson, M.D.	Research Psychiatrist	SMR	NIMH

COOPERATING UNITS (if any)

Clinical Psychopharmacology, St. Elizabeths Hospital, Washington, D.C.

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier findings that 1) sleep deprivation might be beneficial in certain depressed patients and 2) that patterns of average evoked response (AER) were related to individual differences in sleep duration suggested the utility of the AER in clinical sleep deprivation studies. AER data were collected on normal controls, patients with affective illness and medication-free narcoleptics as a comparison. Primary affective disorder patients showed an increase in AER augmenting following sleep deprivation not shown by normal controls.

PHS-6040
(Rev. 10-76)

Project Description:

Sleep EEGs were obtained in two groups of ten college students whose platelet monoamine oxidase activity levels were either in the upper or lower 10 percent of the normal distribution. Their sleep EEGs were then examined for two nights, following a two-night adjustment period. The high MAO group differed from the low MAO group by having density, and fewer total eye movements. This appears to be the first report relating to individual differences in the activity of neurotransmitter-related enzyme to the human sleep stages.

Further studies on the effect of catecholamine precursors on the sleep of individuals with high and low MAO levels are planned.

Publications:

Mendelson, W.B., Buchsbaum, M.S., Murphy, D.L., Wyatt, R.J., and Gillin, J.C.: Platelet monoamine oxidase activity and human sleep. Communications in Psychopharmacology, 2: 539-544, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00037-07 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Genetic Factors in Psychiatric Illness and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clinical Psychophysiology	BP NIMH
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auditory and visual average evoked responses (AERs) were studied in families of patients with affective illness and in a small sample of twins with affective illness. In patients with primary affective illness, affective disorder probands and their siblings showed similar AER amplitude/intensity slopes, as did probands and their spouses. Within the probands' families, the "well" and "ill" relatives were not significantly different with both groups showing significant augmenting (high amplitude/intensity slopes) in comparison with normal groups. This suggests that AER stimulus intensity slope measures and affective disorders are not transmitted by a single genetic factor but that AER may be related to increased genetic vulnerability. Review of the heritability of other electrophysiologic variables also suggested that multiple loci and dominance, epistasis and interactions may be important.

PHS-6040
(Rev. 10-76)

Project Description:

The role of heredity in psychiatric illness is supported by the many studies demonstrating excessively high rates of illness in persons with a genetic relationship to known patients. The clinically variable expression of psychiatric disorders and the difficulty in identification of mildly affected relatives point to the need for biological markers for the genetic vulnerability. The heritability of AER measures combined with their applicability to both ill and well relatives makes them a promising approach. Further, differences have been demonstrated between patients with schizophrenia and various types of affective disorders.

Genetic mechanisms of the AER have been pursued this year in studies of pharmacological effects of amphetamine in normal volunteers. Four pairs of Mz twins have so far been tested on placebo controlled double-blind trials. One twin received two placebo test days to assess test-retest reliability in addition. Data is currently under analysis.

Publications:

Buchsbaum, M.S. and Gershon, E.S.: Genetic factors in EEG, sleep and evoked potentials. In Davidson, J., Davidson, R. J. and Schwartz, G. E. (Eds.): Human Consciousness and Its Transformations. New York, Plenum Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00038-05 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Laterality, Psychopathology and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clin. Psychophysiology	BP NIMH
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Visual AERs from the left and right temperoparietal and occipital cortex were recorded using visual stimuli presented to the left and right hemiretinal fields and to both fields simultaneously. Subjects included normal controls and patients with acute schizophrenia, affective illness and right and left temporal lobectomies for epilepsy. One patient with partial agenesis of the corpus callosum was also studied. Differences in AER caused by attention were noticed especially in the left temperoparietal cortex when stimuli were presented separately to each field. Schizophrenic and lobectomy groups showed diminished asymmetry and attentional effects, whereas bipolar affective disorders showed generally enhanced attentional effects. Full visual field stimulation also produced attentional asymmetries, currently under study, in schizophrenics.

PHS-6040
(Rev. 10-76)

Project Description:

Several psychophysiologic and neuroanatomic studies have suggested that a lateralized deficit may occur in schizophrenia. By presenting visual stimuli in the right and left visual field and recording EEG from the left and right hemisphere, AER techniques can take advantage of the anatomical arrangement of the human visual system to study abnormalities in lateralization. In normal subjects, AERs are highly asymmetrical and differ markedly with the task assigned the subject. AERs from the left temperoparietal area seem especially sensitive to changes in the attention given to the stimulus by the subject. Since attentional deficits have been one of the most widely postulated psychological deficits in schizophrenia, the application of this AER technique seemed promising. A group of 16 off-medication, acute schizophrenics and comparison groups of 17 affective disorder patients and 30 normal controls have been tested. As a further comparison, testing of 15 patients with right or left temporal lobectomies has been accomplished.

The stimuli were lights of two intensities, 0.5 and 50 foot-lamberts, presented as semicircles with their centers 2 cm to the right or left of an illuminated fixation spot. Each stimulus could be illuminated separately from behind by a fluorescent tube driven by an Iconix Photostimulator under computer control. Stimuli were presented in a constrained random sequence such that each visual field and each intensity was presented an equal number of times and was preceded by each other condition an equal number of times.

The stimulus control computer program allowed stimulus presentation only during periods when subjects fixated on the central illuminated spot. The subject's fixation was monitored using Mackworth eye movement camera stand optics and a television camera device interfaced with the computer. So long as fixation was maintained, the stimuli were not presented until the subject's gaze was returned to the designated spot.

The EEG was recorded between right and left occipital and temperoparietal areas and the ipsilateral ear, with Cz as ground.

One task requiring attention to visual stimuli and one task requiring that the stimuli be ignored were chosen from the tasks previously studied to provide maximum EP attention effects. The identical sequence of EP stimuli was presented each time. The visual attention task required the subject to count the number of times he observed two consecutive flashes of one intensity followed by two consecutive flashes of the other intensity, regardless of the side of presentation. The distraction test required mental subtraction of seven serially from 2,019, the answer to be reported at the end of the EP run.

Visual EP amplitude was increased when subjects counted the visual stimulus sequences in comparison to EPs elicited by the same stimuli when subjects did mental arithmetic. As observed in the earlier study, the greatest amplitude enhancement occurred for the N120 component and the temporoparietal (W) lead. Only the N120 had a significant increase, and W area increases were greater in all cases than comparable occipital EPs. This effect was especially true for direct pathway EPs (right hemiretina stimulation - right hemisphere recording and left hemiretina - left hemisphere) where the attention enhancement in the W area was approximately twice as large as in the occipital area.

Patients with affective disorders had larger EP amplitude increases with attention than normals or schizophrenics who were surprisingly similar. The temporal lobectomy patients showed the smallest amplitude increases, the only clinical group not to exhibit statistically significant amplitude increases for any lead or side of stimulation.

The largest evoked potential under any condition (1.89 microvolts) was in affective disorder patients for the direct pathway, attention condition. The smallest EP (.60 microvolts) is for schizophrenics, indirect pathway, inattention condition. Both the L temporal lobectomy patients and schizophrenics have unusually small indirect pathway EPs under attention conditions.

Only 4 of 16 schizophrenics, but 22 of 30 normals, had a low intensity attentional effect of 0.26 microvolts or larger.

The EP results found in the normal controls, psychiatric patients and temporal lobectomy patients reconfirm reports of the saliency of attention effects on the N120 component as well as the existence of different patterns of electrophysiological asymmetry in schizophrenia and affective illness. They are further consistent with the hypothesized left hemisphere deficit in schizophrenia.

Publications:

Buchsbaum, M.S., Carpenter, W.T., Jr., Fedio, P., Goodwin, F.K., Murphy, D.L., and Post, R.M.: Hemispheric differences in evoked potential enhancement by selective attention to hemiretinally presented stimuli in schizophrenic, affective and post-temporal lobectomy patients. In J.H. Gruzelier and P. Flor-Henry (Eds.): Hemisphere Asymmetries of Functions in Psychopathology. Amsterdam, Elsevier, (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00039-05 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Sensory Thresholds and Averaged Evoked Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. C. Davis, M.D.	Sr. Staff, Unit on Studies of Drug Abuse	BP NIMH
	F. K. Goodwin, M.D.	Chief, Clin. Psychobiology Branch	CP NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP NIMH
	D. L. Murphy, M.D.	Chief, Clin. Neuropharmacology Branch	CN NIMH
	M. Webster	Research Assistant	CP NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch; Psychobiology Branch, DCBR, NIMH Dept. of Physiology, George Washington University

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.3	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Average evoked responses (AER) to varying intensities of electrical stimulation to the forearm and subjective ratings of these stimuli have been studied in 32 age and sex matched normal volunteers. Naloxone and aspirin and somatosensory AERs were also studied in normal volunteers.

PHS-6040
(Rev. 10-76)

Project Description:

Individuals who were relatively pain tolerant as indicated by psychophysical shock ratings using signal detection analysis show AER to electrical stimuli which decreased in amplitude with increasing stimulus intensity (reducing).

The discovery of opiate like peptides (endorphins) in brain raised questions as to their physiological role in pain perception and even led some to speculate on their role in affective regulation. Using somatosensory EPs and signal detection psychophysics, we were the first to report a hyperalgesic effect of the opiate blocker, naloxone, in man. We subsequently noted that the opposite of the specific EP augmenting effect caused by naloxone was produced by morphine in a series of controlled and double-blind studies.

Recent evidence points to the production by the pituitary of a prohormone (called pro-opiocortin) which contains the peptide sequences of both adrenocorticotropin (ACTH) and beta-lipotropin (a possible precursor for beta-endorphin). This discovery has been followed by the reports of Krieger that plasma beta-lipotropin levels parallel the diurnal variations in plasma ACTH, rising in the early morning and falling in late afternoon and evening. Thus, if beta-lipotropin functions as a precursor for beta-endorphin and further if beta-endorphin plays a role in pain appreciation, then there should be a diurnal variation in pain sensitivity. To test this hypothesis, we reanalyzed data previously collected.

A group of 35 normal subjects had somatosensory evoked potentials (EPs) recorded and were tested on a psychophysical pain judgment task in morning and afternoon sessions. Subjects tested in the morning were found to be significantly less pain sensitive than those tested in the afternoon on psychophysical judgments. EP measures were consistent with decreased sensitivity in the morning. In a second experiment, a group of 12 normal subjects also received naloxone (2mg, iv) in a double-blind trial, 6 in the morning and 6 in the afternoon. Naloxone hyperalgesia effects on the EP were present in the morning and absent in the afternoon. Our results are consistent with the hypothesis that endorphins at least partially mediate diurnal variations in pain appreciation.

We have previously reported that patients whose visual EPs increase rapidly in amplitude with increasing stimulus intensity ("augmenters") are more likely to be bipolar and more likely to show a positive clinical response to lithium.

This year, somatosensory EPs were recorded in 27 hospitalized inpatients with primary affective illness both during a medication-free baseline period and again after 2 or more weeks of lithium carbonate administration. Mood ratings by nurse-raters were obtained daily during the double-blind placebo controlled study. The early (20-50 msec) and late (100-200 msec) components of the somatosensory evoked potential were found to differ both in their change with lithium and their efficacy for prediction of drug response. The early components which include the primary somatosensory evoked potential showed larger amplitudes when patients were on lithium for nearly every patient studied. However, the amplitude increase was equal for clinical responders and nonresponders, and for bipolars and unipolars. In contrast, the magnitude of late components of off-medication EPs predicted subsequent clinical response to lithium. As was previously reported for visual EPs, in this independent sample of patients we found that individuals whose somatosensory EPs showed marked increases in amplitude with increasing stimulus intensity were more likely to respond to lithium. A comparison of the bipolar/unipolar criterion with an EP criterion revealed that polarity successfully identified more responders, but the EP successfully identified more nonresponders, overall accuracy being approximately equal. Several successful small scale replications of this EP effect suggest the possible utility of a larger clinical trial.

In a study of aspirin, somatosensory EP amplitude and amplitude/intensity slopes paralleled psychophysical rating changes with aspirin, both techniques confirming aspirin analgesia. The statistical strengths of the two techniques, as measured by the paired t values comparing aspirin and placebo were similar, being 2.69 for the nonparametric analogue of d' and 2.86 for the area Cz-N120 amplitude/intensity slope.

Individual differences in analgesic effects of aspirin were reflected in significant correlations between the magnitude of the changes associated with aspirin in psychophysical and EP measures. Variability in the analgesia effect of aspirin was reduced by choosing individuals with typical triphasic EPs, and more consistent effects observed than those achieved with the psychophysical measure used.

It is of interest that naloxone, a drug which showed no total group effect on the psychophysical measure, produced augmenting on the N120 component in pain tolerant individuals, suggesting that individual differences in endogenous opiates might be reflected in the P100-N120 complex (Buchsbaum et al., 1977). The grouping observed here of aspirin effects with endogenous opiate effects is intriguing. Further development of EP techniques with measurement of primary P30 and P50 components and testing of a greater range of analgesics and neurotransmitter strategies as well may be helpful in increasing the neurophysiological/neurochemical correlations.

Publications:

Buchsbaum, M.S., and Davis, G.C.: Application of somatosensory event related potentials to experimental pain and the pharmacology of analgesia. In Lehmann, D. and Callaway, E. (Eds.). Human Evoked Potentials: Applications and Problems. New York, Plenum Press, 1979, pp. 43-54.

Buchsbaum, M.S.: Neurophysiological studies of augmenting/reducing. In Petrie, A. (Ed.): Individual Differences in Pain and Suffering. Chicago, Univ. of Chicago Press, 1979.

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Naloxone decreases diurnal variation in pain sensitivity and somatosensory evoked potentials. Life Sciences, 23:1449-1460, 1978.

Buchsbaum, M.S., Lavine, R.A., Davis, G.C., Goodwin, F.K., Murphy, D.L., and Post, R.M.: Effects of lithium on somatosensory evoked potentials and prediction of clinical response in patients with affective illness. In Gershon, S., Kline, N.S., and Schou, M. (Eds.): International Lithium Conference: Controversies and Unresolved Issues. Excerpta Medica, ICS Series, (In Press).

Davis, G.C., Buchsbaum, M.S. and Bunney, W.E., Jr.: Analgesia to painful stimuli in affective illness. Am. J. Psychiat. (In Press).

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Research in endorphines and schizophrenia. Schizophrenia Bulletin, (In Press).

Gillin, J.C., Sitaram, N., Mendelson, W.S., Buchsbaum, M.S., Moore, A.M., Jacobs, L.S. and Wyatt, R.J.: Cholinergic modulation of sleep, growth hormone and analgesia in normal man. In Jenkin, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, (In Press.).

Davis, G.C., Bunney, W.E., Jr., Buchsbaum, M.S., DeFraites, E. G., Duncan, W., Gillin, J.C., van Kammen, D.P., Kleinman, J., Murphy, D.L., Post, R.M., Reus, V. and Wyatt, R.J.: The use of narcotic antagonists to study the role of endorphins in normals and psychiatric patients. In Usdin, E. and Bunney, W.E., Jr. (Eds.): Endorphins and Mental Health Research. New York, MacMillan, (In press).

Haier, R.J., Rieder, R.O., Khouri, P.J. and Buchsbaum, M.S.: Extreme MMPI scores and the research diagnostic criteria: Screening males for psychopathology. Archives of General Psychiatry, (In Press).

Lipper, S., Murphy, D.L., Slater, S. and Buchsbaum, M.S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology, (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00040-04 BP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Amphetamine Pharmacokinetics and the Average Evoked Response in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clinical Psychophysiology	BP	NIMH
OTHER:	M. H. Ebert, M.D.	Chief, Section on Exper. Therapeutics	LCS	NIMH
	G. L. Brown, M.D.	Medical Officer, Unit on Childhood Mental Illness	BP	NIMH
	J. L. Rapoport, M.D.	Staff Psychiatrist, Unit on Childhood Mental Illness	BP	NIMH
	A. J. Sostek, Ph.D.	Guest Worker, Section on Clinical Psychophysiology	BP	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Science, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of amphetamine on the average evoked response (AER) is being studied in hyperactive children. Blood amphetamine levels will be correlated with AER measures in an attempt to understand individual differences in response. The AER is only one phase of a larger project reported elsewhere. In an effort to demonstrate possible perceptual response stereotypy associated with amphetamine, single evoked response trials are being collected for both visual and auditory stimuli. These single trials are then being analyzed for signal/noise and trial-to-trial similarity using newly developed signal processing techniques.

Project Description:

Previous research has demonstrated changes in average evoked responses (AER) with administration of amphetamine in children and in patients with affective disorders. Individual differences are prominent both in behavioral response as well as the AER; some individuals are aroused and others show sedation or no effect and AERs both predict and parallel these differences. We examined the behavioral, cognitive and EP effects of a single dose of amphetamine in 14 normal prepubertal boys, 15 age matched hyperactive boys, two groups of adults tested at different dosages and in 16 adult Mz twin pairs (see Project Z01 MH 00037-07 BP) in a double blind study. When amphetamine was given, the normal boys showed a marked decrease in motor activity, improved vigilance performance and EP shifts away from augmenting. Very similar results, especially for EPs, were obtained in hyperactive children. The similarity of the response to amphetamine in normal and hyperactive children cast doubt on the concept of a clinically specific or "paradoxical" response to stimulants in this syndrome. For both vigilance and EP data, age affected EP results more than diagnostic group membership; age changes suggested delayed maturation in hyperactive children.

During the next year, data analysis and summary will be done on the multiple groups of individuals tested.

In a related project, an improved and more powerful measure of vigilance based on the continuous performance task was developed, using signal detection theory. Reanalysis of the data comparing hyperactive and normal children confirmed the above findings. These new measures proved reliable individual traits in test-retest trials. Given the reliability, sensitivity to amphetamine and age effects, this new measure formed the basis of a screening study for attentional dysfunction. Four hundred normal college students were tested and the upper and lower 5% recalled for psychiatric histories, childhood hyperactivity assessment, neurological, psychological and psychophysiological tests. A high followup rate was obtained (93% of the 43 high-risk subjects) and the studies completed by a team of blind testers. Data is currently under analysis and will be reported next year.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00111-05 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Drug Abuse Studies/Stimulant Abuse/Opiate Abuse
Formerly: Development of a Drug Abuse Treatment and Research Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

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Glenn C. Davis, M.D.,	Staff Psychiatrist, Unit on Studies of Drug Abuse	BP	NIMH

Other:

Robert M. Post, M.D.,	Chief, Section on Psychobiology	BP	NIMH
Michael Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH
Gerald L. Brown, M.D.	Medical Officer, Unit on Child Studies	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward a basic understanding of the mood alterations induced by drugs of abuse. Several areas are under investigation: The high produced by intravenous amphetamine and cocaine, chronic behavioral states caused by chronic use of stimulant drugs; neurological effects of chronic use of stimulant and opiate drugs.

PHS-6040
(Rev. 10-76)

Objectives

1. Drugs of abuse such as cocaine and amphetamine produce profound alterations in mood. There are major differences in acute and chronic administration. Our studies are designed to obtain a neurochemical understanding of these alterations.

2. Many individuals who use drugs chronically have motor and behavioral abnormalities. We are studying the causes and treatments for these abnormalities.

3. The chronic use of stimulant medications appears to produce depressions that mimic major depressive illness. Through study of the effects of the drugs, we hope to learn more about the etiology of affective illness.

Methods Employed

1. Patients who meet criteria for drug abuse stimulant-type, or drug abuse opiate-type are admitted to the Clinical Center Wards 2-West or 3-West for study and treatment.

(1.1) Treatment includes drug withdrawal and detoxification, if necessary, and careful medical evaluation for complications of chronic drug use (for example, hepatitis). Patients have the opportunity to become involved in intensive individual psychotherapy. Psychotherapy is directed toward individual needs and cognitive understanding of the function that drug use serves in the individual. Careful nursing intervention is offered as well, especially help directed towards dealing with common life-style problems manifested by a drug user in the ward situation.

(1.2) Historical and daily behavioral data. We obtain extensive histories of drug use including drug preference, frequency, chronicity and subjective effects of drugs. Other data are obtained in the ward situation utilizing nurse ratings of mood and behavior.

(1.3) Infusion studies. Many of the drug abuse studies center around the administration of various pharmacological agents. Amphetamine or cocaine are administered in non-tolerant patients (in doses that are less than the patient's previous habit). Behavior is rated by trained investigators along various dimensions; blood and urine are collected for amphetamine or cocaine levels, various catecholamines and their metabolites. Lithium or alpha-methyl-para-tyrosine (AMPT) is administered in placebo-controlled double-blind fashion to study the alterations of subjective effects produced by these compounds.

(1.4) Case reports. Patients suffering a variety of major side effects of drugs of abuse are admitted for exhaustive biochemical and behavioral studies.

Major Findings

1. Parkinsonism is a well recognized common side effect of drug treatment which either depletes central catecholamines or blocks dopamine receptors. Drug-induced parkinsonism usually develops over several weeks. When the

offending drug is discontinued, remission is often gradual taking several weeks. This Unit has studied the acute development of parkinsonism in a young man subsequent to his parenteral use of a meperidine congener. This patient's symptoms persisted for eighteen months, but we treated him successfully with bromocryptine, a potent dopamine receptor agonist. After discharge from NIH care and referral to a private psychiatric follow-up, this patient committed suicide. His family wished that the autopsy be done at NIH. Neuropathological examination revealed specific structural damage to the substantia nigra. The levels of biogenic amines and metabolites in the CSF, the clinical symptomatology, the therapeutic response to dopamine receptor stimulation and the microscopic evaluation of the brain at necropsy are all consistent with a drug-induced damage to aminergic neurons in the substantia nigra which resulted in a parkinsonian syndrome.

To our knowledge, this is the only case of drug-induced disease with pathogenic findings which mimic those of Parkinson's Disease, particularly in the appearance of Lewy bodies. This observation suggests that Lewy bodies are the result of destruction of cells in the substantia nigra rather than being representative of a specific etiologic factor in Parkinson's Disease.

2. In collaboration with the Unit on Childhood Mental Illness, we have studied the acute effects of methylphenidate in a young adolescent hyperactive child. This child abused methylphenidate. We found in controlled ward studies that he did not manifest typical euphoric or other subjective effects of high dose methylphenidate that is experienced by older drug abusers.

3. Much of the work of this project was deferred in this fiscal year. It is expected that during 1979-1980 the stimulant project may begin again.

Significance to Biomedical Research

1. Drug abuse is a significant problem. A basic understanding of the mechanisms of action of drugs of abuse could lead to pharmacological agents useful in prevention, detoxification, withdrawal and long-term treatment.

2. The chronic use of stimulant and opiate medication produces clinical states similar to major affective illness. Thus, chronic drug use may provide a limited model of affective illness.

3. Motor side effects occur with most drugs of abuse. The linkage between motor effects and mood effects is an important one to the understanding both of affective illness and drug abuse.

Proposed Course

Studies are ongoing at this time.

Publications

Goyer, P., Davis, G.C., Rapoport, J.: Abuse of prescribed stimulant medication by a hyperactive child. J. Am. Acad. Child Psychiatry 18: 170-175, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00112-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Endorphin Research in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Division of Special Mental Health Research, NIMH

LAB/BRANCH

Biological Psychiatry Branch

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Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endogenous opiate-like substances (endorphins) have recently been discovered. These substances function both as neurotransmitters and as neuromodulators within the central nervous system. Alterations in the function of endorphin mediated behaviors may play a role in mental illness. We are studying the action of endorphins on mood, memory, hormones, and learning. This Unit has tested the efficacy of narcotic antagonists in the treatment of affective illness and schizophrenia and will be studying the pharmacological and possibly therapeutic effects of beta-endorphin in man.

PHS-6040
(Rev. 10-76)

Objectives

In this project we are seeking to understand the role of endorphins in normal physiology and in mental illness.

Methods Employed

Patients admitted on 2-West, 3-West, 4-East and at St. Elizabeth's are acutely administered narcotic antagonists and endorphins intravenously in double-blind placebo-controlled fashion to determine whether endorphins play a role in these illnesses. Clinical studies with the narcotic antagonist naltrexone are ongoing on 4-East.

Major Findings

1. A second study of the effects of naloxone in schizophrenics was carried out at St. Elizabeth's Hospital. Preliminary analysis of data revealed that 15 mg of naloxone i.v. acutely (for one hour) improved the "thought disorder" of the BPRS. This is a replication of our earlier study with fourteen schizophrenics.

2. Ten manic patients were tested using 20 mg of naloxone intravenously. While one of eight patients receiving naloxone in an earlier study showed a dose-dependent improvement in the symptoms of "pressure of speech" and "pressure of thought," no manic patient in this study showed such an effect. In fact, no significant improvement was seen on BPRS and Beigel-Murphy Manic Rating Scales.

3. Fifteen schizophrenic and affectively ill patients were administered between 2 and 15 mg of beta-endorphin. No clinical improvement was noted. Data analysis of rating scales is taking place at this time.

4. A multi-national WHO study has been initiated to study the effect of naloxone in mania and schizoprenia.

5. Five patients have received the oral opiate antagonist naltrexone for up to one month. No clear cut clinical improvement has been seen. This study is continuing in order to provide a more definitive answer to whether narcotic antagonists may be useful in schizophrenia.

Significance to Biomedical Research

Endorphins may play an important physiological role in man. Results to date are suggesting a weak effect of naloxone at 15 mg doses in unusual thought content and conceptual disorganization in schizophrenics. Studies of naltrexone are not complete and results to date in mania have failed to demonstrate an effect of naloxone on mood or activity.

Proposed Course

All studies are ongoing.

Publications

Davis, G.C. and Bunney, W.E., Jr.: Introduction: Review of clinical studies. In Usdin, E., Bunney, W.E., Jr., and Kline, N.S. (Eds.): Endorphins in Mental Health Research. New York, McMillin, 1979, pp. 601-602.

Davis, G.C., Bunney, W.E., Jr., Buchsbaum, M.S. et al.: The use of narcotic antagonists to study the role of endorphins in normals and psychiatric patients. In Usdin, E., Bunney, W.E., Jr., and Kline, N.S. (Eds.): *Endorphins in Mental Health Research*. New York, McMillan, 1979, pp 393-406.

Davis, G.C. and Bunney, W.E., Jr.: Studies of the role of endorphins in normals and psychiatric patients. Conference of the Combined Clinical Staffs, NIH, *Ann. Intern. Med.*, in press.

Pert, A., Pert, C.B., Davis, G.C., and Bunney, W.E., Jr.: Opiate peptides and brain function. In van Praag, H. (Ed.): *Handbook of Biological Psychiatry*. In press.

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Research in endorphins and schizophrenia. *Schizophr. Bull.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00113-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychophysiological Studies of Pain and Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Biological Psychiatry Branch

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2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal volunteers, affectively ill, and schizophrenic patients participate in studies which use psychophysical and psychophysiological techniques to further our understanding of these illnesses and to determine the effects on perception of therapeutic drugs.

Objectives

1. To understand normal pain perception.

2. To understand how alterations in pain perception in affective illness and schizophrenia may give clues to other biological alterations in these illnesses.

3. To develop measures which will predict drug response among patient subgroups.

Methods Employed

1. Studies of affectively ill and schizophrenic patients hospitalized on the wards of the National Institute of Mental Health.

2. Normal volunteers are studied both in inpatient and outpatient experiments.

3. Procedures include visual, auditory, somatosensory evoked potential measurements; h-reflex; continuous performance measures; somatosensory stimulation with psychophysiological pain rating tests.

4. Double-blind, placebo-controlled drug administration.

5. Other psychosocial manipulations (e.g. attention).

Major Findings

1. A group of thirty-five normal subjects had somatosensory evoked potentials recorded and were tested on a psychophysical pain judgment task in the morning and afternoon sessions. Subjects tested in the morning were found to be significantly less pain sensitive than those tested in the afternoon on psychophysical judgments. E.P. measures were also consistent with pain insensitivity in the morning. In a second experiment, a group of twelve normal subjects also received naloxone in a double-blind trial, 6:00 in the morning and 6:00 in the evening. Naloxone hyperalgesia effects on the evoked potential were present in the morning and absent in the afternoon. Our results are consistent with the hypothesis that endorphins at least partially mediate diurnal variations in pain appreciation.

2. Complaint of pain is a prominent symptom in affective illness. Seventy-three affectively ill patients and forty-eight age and sex matched normal controls participated in a pain rating procedure consisting of single shocks administered at 2.5 second intervals. Male unipolar and bipolar depressed patients were significantly less pain sensitive than controls while female depressed patients were not. Bipolar depressed male patients demonstrated decreased sensory discrimination while unipolar patients only demonstrated a tendency to call higher intensity stimuli unpleasant. Both male and female manic patients were less pain sensitive across most measures.

3. Using the pain measurement paradigm described, thirty-two normal subjects were administered aspirin or placebo. An analgesic effect of aspirin

was demonstrated both for psychophysical pain ratings as well as for evoked potentials. Furthermore, individual differences in analgesic effects of aspirin were a function of the pain sensitivity of the subjects.

4. Seventeen schizophrenic individuals were found significantly more pain tolerant than age and sex matched normals. Five schizophrenics tested after chronic naltrexone and placebo demonstrated hyperalgesia and normalization of their evoked potentials on naltrexone.

Significance to Biomedical Research

1. The demonstration of pain tolerance to experimental stimuli among affectively ill and schizophrenic patients suggests a possible link with endogenous opiate-like substances. Research in this area will contribute toward our understanding of affective illness and possible new therapeutic treatments.

2. Endorphins play an important physiological role in pain regulation in man. It appears that the diurnal release of endorphins may be linked to a physiological variation in pain sensitivity during the day.

Proposed Course

All studies are ongoing.

Publications

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Analgesia to painful stimuli in affective illness. *Am. J. Psychiatry*, in press.

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Buchsbaum, M.S. and Davis, G.C.: Application of somatosensory event related potentials to experimental pain and the pharmacology of analgesia. In Lehmann, D. and Calloway, E. (Eds.): *Human Evoked Potentials*. New York, Plenum Press, pp. 43-54, 1979.

Buchsbaum, M.S., Levine, R., Davis, G.C., Murphy, D.L., Goodwin, F.K., and Post, R.M.: Effects of lithium on somatosensory evoked potentials and prediction of clinical response in patients with affective illness. In Gershon, S., Kline, N.S., and Schou, M. (Eds.): *International Lithium Conference: Controversies and Unresolved Issues*. New Jersey, Excerpta Medica, in press.

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Pain and psychiatric illness. In Ng., L. and Bonica, J.J. (Eds.): *Pain, Discomfort and Humanitarian Care*. North Amsterdam, Elsevier, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00070-06 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Interactions in the Acute Psychoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Clinical Center Rehabilitation	NIH

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SECTION
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12.0	6.0	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on longitudinal clinical and biological evaluation for the treatment of mania, depression, and schizoaffective illness. In double-blind clinical trials, we have found evidence that the dopamine receptor active agents piribedil and pimozide possess clinical efficacy in depression and mania, respectively. The anticonvulsant carbamazepine is useful in the treatment of mania and may be of some prophylactic use in both manic and depressive phases, including in some lithium non-responders. A variety of neurotransmitter amines and their metabolites are measured in patients and controls. Preliminary data indicate that CSF norepinephrine is elevated, while its biosynthetic enzyme DBH is decreased in mania compared to controls. Approaches to neuroendocrine and polypeptide function in patients and volunteers are being pursued. A psychopharmacology laboratory is maintained for the study of animal models of psychopathology. Kindling and cocaine sensitization have proven most useful in the attempt to examine underlying mechanisms of behavioral pathology. The interaction of biological changes with psychological and cognitive alterations in the acute psychoses are considered in developing new research and treatment strategies for the affective psychoses.

I. Project Description:

A. Objectives: The project is engaged in the multidisciplinary, longitudinal study and treatment of patients with a spectrum of acute psychoses, particularly involving mood disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interactions.

B. Methods Employed:

1. Patients who meet formal Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. A rating scale for atypicality of depression has been developed with Dr. Edward Silberman in order to more adequately differentiate and subtype even the patients who meet RDC for primary depressive illness, yet appear to form a heterogeneous group.

2. Normal volunteers are also admitted to provide control data, as well as to assess the relationship of selected biological and psychological measures directly in normals. Forty-three normal volunteers have already been studied with lumbar punctures and a battery of other biochemical, physiological, and psychological tests.

3. During an initial drug-free interval, patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. These also include EEG monitored sleep, averaged evoked potentials, the Halstead Categories test, and other tests of cognitive function. Treatment and clinical evaluation are begun in individual and group psychotherapy, as well as ongoing clinical case conferences. Behavioral data are collected in a double-blind fashion twice daily, both by a trained nursing team and by patient self-ratings. Nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) and Hamilton Depression Inventory three times weekly.

4. Motor activity is measured continuously every 15 minutes utilizing a miniaturized activity monitor developed by Dr. Theodore Colburn. Temperature and self-ratings of mood are measured every two hours to study circadian and infradian biological rhythms.

5. A "life chart" technique has been developed to plot number, severity, and interval between affective episodes, so that the development, recurrence, and progression of the illness can be assessed.

6. Clinical and neuroendocrine parameters are evaluated following administration of apomorphine, thyrotropin releasing hormone, arginine, LHRH, and hypertonic saline.

7. The degree of hypothermia following apomorphine is measured to provide an index of hypothalamic dopamine receptor function.

8. Amine metabolites and urinary free cortisol are systematically evaluated utilizing daily 24-hour urinary collections during clinical state changes on and off medication.

9. Amine metabolites, electrolytes, and peptides are also measured during ill and well intervals in blood and CSF.

10. Both routine and experimental compounds are evaluated during double-blind clinical trials. Routinely used drugs including tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics are studied, not only for clinical efficacy, but to further understand their mechanisms of action.

11. Several experimental drugs with relatively specific receptor effects are also studied. A dopamine receptor agonist and antagonist, piribedil and pimozide, are used to dissect the role of dopamine in affective illness. Clonidine, an α-adrenergic agonist, and propranolol, a beta blocker are also used.

12. Peptide mechanisms are explored using acute strategies with naloxone, ACTH, and Organon 2766, and chronic treatment with the vasopressin analog DDAVP.

13. The anticonvulsant carbamazepine is given acutely and chronically to patients with affective illness as a new treatment protocol.

14. The paradoxical acute antidepressant effects of one night's sleep deprivation in severely depressed patients is examined and possible underlying neurobiological and neuroendocrine changes are investigated.

15. A rodent and primate behavioral pharmacology laboratory is maintained in order to develop new research techniques in the longitudinal study of biobehavioral interactions. Particular focus is given to the study of chronic cocaine-induced behavioral pathology and the underlying changes produced by repetitive stimulation of the amygdala (electrical kindling).

C. Major Findings:

1. A New Treatment Strategy for Manic-Depressive Illness: Use of the Temporal Lobe Anticonvulsant Agent Carbamazepine (Tegretol). A detailed series of clinical and biological studies of carbamazepine have been initiated by the Section on Psychobiology in collaboration with Drs. James Ballenger and William Bunney, Jr. The clinical trial of carbamazepine was undertaken in light of the consistent positive effects on psychiatric symptoms in patients with psychomotor epilepsy; the predominantly temporal lobe and limbic effects of the drug; its ability to inhibit limbic seizures, kindling, and other model psychoses; and preliminary data in open trials in Japan that the drug might have some efficacy in manic-depressive patients. Initial data in our double-blind, clinical evaluation indicate that the drug had marked antimanic effects in four of nine patients. To date, seven of nine patients have shown evidence of an antimanic response, with four also showing evidence of relapse upon pla-

cebo substitution. These positive responses have been observed in some patients who have been previously noted to be lithium non-responders. In addition to acute antimanic effects which are significant for the group as a whole ($p < .05$), several patients have been maintained on carbamazepine for longer periods of time with evidence of sustained or prophylactic effects against their recurrent mood disorder. Our preliminary data in 13 patients also suggests that carbamazepine may have some acute and prophylactic effects in the depressed phase of illness.

Whether the predominantly limbic and temporal lobe anticonvulsive effects of carbamazepine are related to its psychotropic effects in manic-depressive illness is unknown. Biochemical studies in man indicate that carbamazepine may affect several neurotransmitter systems. Cerebrospinal fluid accumulations of homovanillic acid (HVA), the dopamine metabolite, were significantly decreased in patients treated with carbamazepine. The possible effects of carbamazepine on dopaminergic activity in relation to the antimanic and antidepressive effects of the drug remain to be elucidated. Cerebrospinal fluid γ-aminobutyric acid (GABA), measured in collaboration with Dr. Theodore Hare, did not change significantly. Patients did not show a consistent alteration in the urinary norepinephrine metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) during carbamazepine treatment. Cerebrospinal fluid norepinephrine, measured in collaboration with Dr. Ray Lake, decreased in four manic patients treated with carbamazepine and showed little change or increased in depressed patients treated with the drug.

If the preliminary evidence of carbamazepine's acute and prophylactic effects in manic and depressive illness continue to be substantiated in further work, these data would suggest that it could play an important role in the treatment of recurrent affective illness. It appears useful in some lithium non-responsive patients and may be of use in those unable to take lithium carbonate because of side effects. It may also have a role in patients who would otherwise require neuroleptic treatment in addition to lithium carbonate for management of recurrent manic or schizoaffective mood disturbances. In the wide experience with the drug in patients with epilepsy, there has been no reported incidence of tardive dyskinesia which is common with chronic neuroleptic treatment. In addition, because of its spectrum of therapeutic effects in patients with psychomotor epilepsy and in patients with affective illness, the drug may provide new clinical and theoretical leads to the inter-relationships between these syndromes and their possible underlying neurochemical and biological substrates.

2. Noradrenergic Mechanisms in Affective Illness.

a. Clonidine is a relatively specific α-noradrenergic agonist. In low doses it inhibits the firing of the locus coeruleus. We are continuing a trial of clonidine in mania to investigate whether low doses of this agonist might be associated with antimanic effects.

b. Propranolol. In collaboration with Drs. Neal Cutler and Al Lewy, we are studying the effects of the β-adrenergic receptor blocking compound propranolol in mania and in psychotic depression. Our first depressed patient showed a decrease in depression and psychosis ratings during blind administration of propranolol and exacerbation on placebo substitution. High doses of propranolol also inhibited the night-time secretion of melatonin.

c. CSF Norepinephrine. Manic patients studied in collaboration with Dr. Ray Lake continue to show significantly higher baseline and probenecid-induced accumulations of CSF norepinephrine than do depressed patients, neurological controls, or normal volunteers. These increases in CSF norepinephrine were observed in some manic patients where complete bed rest was clearly maintained, although an effect of psychomotor activity remains to be ruled out.

d. Dopamine β-Hydroxylase (DBH). Although CSF levels of norepinephrine were significantly higher in manic patients, its biosynthetic enzyme DBH was significantly reduced in the CSF of these patients (measured in collaboration with Dr. Pauline Lerner). These data indicate that the increases in CSF norepinephrine in mania are not associated with an increased enzyme activity and suggest that altered transport characteristics or blockade of reuptake of norepinephrine could account for the discrepancies between enzyme activity and its end-product, norepinephrine. It is also possible that high levels of norepinephrine feed back and inhibit enzyme activity. The trials of clonidine and propranolol in mania should help clarify whether the increases in norepinephrine are closely associated with the manic process. Dopamine β-hydroxylase levels were significantly higher in bipolar depressed patients than normals. In the depressed population CSF DBH and norepinephrine were positively correlated.

e. Plasma MHPG. In collaboration with Dr. David Jimerson we are measuring plasma MHPG longitudinally during manic and depressive phases of the illness. Preliminary data suggest that MHPG levels are higher during the manic than depressive phase in both plasma and urine.

3. The Involvement of Dopamine in Mania and Depression: Implications for Treatment.

a. Piribedil is a drug which acts in part by directly stimulating dopamine receptors. As such, it is a clinical treatment strategy as well as a research tool in dissecting the possible role of dopaminergic mechanisms in affective illness. Our clinical evaluation of piribedil continues to suggest that several depressed patients show substantial clinical improvement on the drug, while most experience only mild to moderate changes. Fourteen of 19 patients improved, two showed no change, two patients became worse, and one showed the emergence of manic symptomatology during treatment of his depression. Several of the best responders to piribedil have previously been non-responders to routinely used tricyclic antidepressant compounds which are thought to predominantly affect serotonin and norepinephrine mechanisms. These data may indicate a useful role of a dopamine active agent in a subgroup of depressed patients.

b. Low doses of piribedil like those of other direct dopamine receptor agonists may act preferentially on presynaptic dopamine receptors and produce inhibitory effects on behavior. Three of four manic patients have responded to a clinical trial using low doses of piribedil.

c. Pimozide is an antipsychotic drug which is more specific in blocking dopamine receptors than the other commonly used agents. Eight of nine manic patients studied in collaboration with Drs. Frederick Goodwin, William Bunney, Jr., and David Jimerson have shown notable antimanic responses to this compound. These data again are suggestive of a role for dopaminergic hyperactivity in mania and indicate that blockade of dopamine receptors is associated with therapeutic effects in manic patients.

4. Apomorphine-induced Hypothermia. In collaboration with Drs. Neal Cutler and William Bunney, Jr. apomorphine-induced hypothermia has been investigated in man as a strategy for assessing hypothalamic dopamine receptor responsivity. Apomorphine (0.75 mg, subcutaneously) produced significant hypothermic responses at 9:00 p.m. but not at 9:00 a.m. These preliminary data suggest that there may be diurnal differences in hypothermic responsivity to a dopamine receptor agonist challenge. The degree of hypothermic response was not directly correlated with apomorphine-induced changes in mood or side effects. At 9:00 a.m. when there were no significant hypothermic effects, marked increases in growth hormone were observed. These data suggest that different areas of brain mediating dopaminergic responses to nausea (area postrema), prolactin (pituitary), hypothermia (hypothalamus), growth hormone (hypothalamus), sedation, mood, and behavior (possibly limbic and cortical substrates) may differ in their degree of responsivity to a dopamine agonist challenge. This tool can now be systematically applied to studies of manic and depressive phases of the illness, pharmacological treatments, and alterations in diurnal rhythms in man.

Drs. David Jimerson and Alix Rey found that apomorphine-induced growth hormone increases were not significantly different in 14 depressed patients compared to eight controls, although values in three manic patients were markedly reduced compared to either group. These data are compatible with an altered dopaminergic system in mania, possibly dopaminergic hyperactivity leading to dopamine receptor hyposensitivity.

We are continuing to explore whether apomorphine-induced changes can provide a predictor of clinical and pharmacological responsivity. To date, apomorphine-induced increases in growth hormone do not appear predictive of degree of amphetamine-induced euphoria or the degree of antidepressant response to the direct dopamine agonist piribedil. However, the latency to peak increase in growth hormone following apomorphine was significantly correlated with a variety of measures of amphetamine-induced mood elevation, suggesting that those patients with the slowest growth hormone response to apomorphine were the best responders subsequently to amphetamine.

5. Paradoxical Therapeutic Responses to Sleep Deprivation in Depressed Patients. We are continuing to observe moderate to marked clinical improvement following one night's sleep deprivation in more than half of our depressed patients, particularly those with the most severe depression. This response is not observed in normal volunteers or in those with only mild depressions.

In collaboration with Dr. Chris Gillin and associates, it has been found that baseline measures of EEG monitored sleep adequately predict sleep deprivation response. That is, those patients with an EEG sleep pattern that is most typical of depressed patients, as assessed by discriminate function analysis, had the most robust antidepressant responses to sleep deprivation. In addition, several averaged evoked potential responses (studied with Dr. Monte Buchsbaum) predicted sleep deprivation response. In one depressed patient treated with repeated sleep deprivation, there appeared to be tolerance to the antidepressant effects. These findings, in association with the transient antidepressant response usually lasting only one day, appear to limit the clinical utility of the sleep deprivation procedure.

In collaboration with Drs. James Ballenger, Neal Cutler, William Bunney, Jr., and Elliot Weitzman, we are measuring circadian rhythms in a variety of hormones before, during, and after sleep deprivation-induced mood improvement.

Drs. Neal Cutler and Herb Weingartner are continuing to study cognitive alterations following sleep deprivation in depressed patients and normals. Preliminary data suggest that some aspects of cognitive functioning improve following sleep deprivation, even in the normal volunteers who experience no mood elevation. These data, taken in conjunction with previous findings of state dependent alterations in learning and memory induced by the sleep deprivation procedure, suggest that substantial alterations in cognitive functioning, as well as in mood, can accompany disruption of one night's sleep. Unraveling the mechanisms underlying the paradoxical antidepressant effects of sleep deprivation may lead to new nonpharmacological therapies of depression. A dramatic reversal of the sleep deprivation-induced improvement occurs in many patients following one night's recovery sleep. In a parallel fashion, understanding mechanisms underlying this reversal of sleep deprivation-induced improvement may yield important clues to the nature of the depressive process.

6. Pituitary Adrenal Axis and Opiates in Affective Illness.

a. Higher levels of urinary free cortisol during depression than during mania have been documented in collaboration with Drs. Phil Gold and James Ballenger. In rapid cycling patients marked increases in urinary free cortisol closely coincide with the switch into depression. With Dr. Pauline Lerner, we are studying whether CSF levels of cortisol are increased in depressed patients compared to other psychiatric groups, as well as normals.

b. ACTH. In order to dissect possible central and peripheral effects of ACTH on mood and cognition in depressed patients, we have administered ACTH and an analog (Organon 2766) which is active centrally but does not increase adrenal steroids. Substantial effects on mood have not been noted, although some changes in tests of cognition were observed, including enhancement of processing of emotionally neutral verbal material.

c. Opiate receptor blockade with naloxone. In order to test whether endogenous opiates such as β-endorphin or enkaphlin could be involved in the manic process, the receptor blocker naloxone has been administered to acutely manic patients. To date, in collaboration with Drs. Glen Davis, William Hamilton, and William Bunney, Jr., little evidence for such an effect has been observed.

d. To investigate the integrated function of the hypothalamic-pituitary axis in depression and mania we have performed 90-minute arginine infusions followed by TRH and LH-RH. The prolactin and growth hormone responses to TRH and the LH and FSH responses to LH-RH are measured in collaboration with Drs. James Ballenger, Phil Gold, and Samuel Yen.

7. Cerebrospinal Fluid Studies as an Approach to the Central Nervous System Neurotransmitter and Modulator Function in Man.

a. Normal Volunteers. Forty-three normal volunteers have received lumbar punctures as part of our protocol to study possible biological correlates of normal physiology, cognition, and personality. These studies, as well as many reported below, have been conducted by Dr. James Ballenger, in collaboration with Drs. Frederick Goodwin, William Bunney, Jr., Edna Gordon, and many other collaborators.

b. Clinical Methodological Issues. We have explored a variety of factors that could influence CSF amine metabolism. Increases in motor activity produce significant increases in 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). Preliminary data, analyzed in collaboration with Dr. Anna Wirz, suggest that there may be annual rhythms in neurotransmitter function and that values of CSF probenecid may also have an annual rhythm. In our depressed patient population age did not significantly correlate with baseline measures of CSF 5HIAA, HVA, MHPG, VMA, and norepinephrine. In contrast, there do appear to be substantial sex differences in amine metabolites; both 5HIAA and HVA accumulations following probenecid are higher in women than men and post-menopausal, amenorrheaic depressed females have higher 5HIAA than menstruating females (studied in collaboration with Drs. Phil Gold and David Jimerson).

c. CSF Norepinephrine and Its Metabolites. As noted above, CSF norepinephrine, measured directly in collaboration with Dr. Ray Lake, is higher in mania than in depression or in normal controls. While recent mass fragmentographic assays do not support initial findings of higher levels of MHPG in mania than in depression, as measured by the gas chromatographic method, the severity of mania correlates positively with CSF MHPG ($r = .59$, $n = 13$, $p < .03$) and CSF MHPG is negatively correlated with severity of depression.

d. Dopamine Metabolites in CSF. Baseline HVA levels are significantly higher in manic than depressed patients ($p < .04$), although accumulations following probenecid are not significantly different between the two groups. However, following neuroleptic treatment, probenecid-induced HVA accumulations are significantly higher in manic patients than in other populations, again suggesting altered dopaminergic mechanisms during the manic state.

e. Serotonin Metabolites. While baseline 5HIAA is significantly higher in manics than in depressed patients, probenecid-induced accumulations again do not differentiate the two groups. 5-Hydroxyindoleacetic acid values in a given patient remain relatively stable over time. In 11 acutely depressed patients we have studied during an improved drug-free state, 5HIAA values were correlated ($r = .74$, $p < .01$). We are now attempting to replicate the findings by other groups that those patients with low 5HIAA respond best to tricyclic antidepressants and other strategies which increase serotonergic tone. In our studies both acute and chronic administration of the tricyclic antidepressants amitriptyline and imipramine produce statistically significant decreases in 5HIAA, presumably on the basis of a feedback mechanism.

f. GABA. CSF levels of GABA are not significantly different among 40 normal volunteers, 14 depressed patients, and seven manics, studied in collaboration with Dr. Ted Hare. These data did not support a GABA deficiency hypothesis of mania, as postulated by others.

g. CSF Calcium. Although levels of total calcium in CSF are not significantly different among depressed, manic, and control populations, CSF calcium tends to decrease when depressed patients improve following electroconvulsive therapy, sleep deprivation, lithium treatment, or spontaneous switches into mania. We have recently focused on measurements of ionized calcium in CSF and have documented that approximately 77% of calcium appears in CSF in the ionized form, studied in collaboration with Drs. William Hamilton, David Jimerson, James Ballenger, and William Hicky. Preliminary analysis of the data indicates that ionized calcium is not significantly different among psychiatric patients and normal groups.

h. CSF Approaches to Peptide Function in Affective Illness. A number of peptide hormones are now measureable in CSF, some of which are thought to be directly secreted into the CSF as part of an active regulatory pathway. Many of these peptide hormones are also thought to have a neurotransmitter or neuromodulatory role in addition to their peripheral target organ effects. Measurable quantities of ACTH in patient populations and controls have been found in CSF in collaboration with Dr. John Kendall.

In collaboration with Drs. Phil Gold and G.L. Robertson, CSF vasopressin has been analyzed by radioimmunoassay and preliminary data are consistent with the suggestion that values are lower in 12 bipolar depressed patients than in 12 manic patients and normals and higher in manics than normals. Preliminary results of the vasopressin response to hypertonic saline suggest that the sensitivity of the central vasopressin receptor is increased in mania as compared to depression. Lithium increased the sensitivity and threshold of the central receptor, and carbamazepine, in two patients, decreased the sensitivity. Effects of drug treatments including lithium, carbamazepine, and DDAVP on vasopressin in CSF are being analyzed.

CSF somatostatin, measured in collaboration with Dr. S. Reichlin and Phil Gold, indicate the values in depressed patients are in the normal range. In one rapidly cycling patient studied twice during depression and mania, somatostatin values were 133% higher during mania than during depression.

In collaboration with Dr. Ken Becker, we have documented that calcitonin is measurable in the CSF of the majority of healthy volunteers, as well as several psychiatric populations. Preliminary analysis of the data suggests the possibility that thyrocalcitonin may be lower in some manic and psychotic patients compared to depressed patients and normal controls.

Several components of the angiotensin system are likewise measurable in the CSF of man. Preliminary findings in collaboration with Drs. D. Ganten and Phil Gold indicate higher values for angiotensin I in psychiatric patient populations than in normal controls.

Measurement of these and related peptide hormones by sensitive radioimmunoassays holds the promise of clarifying their normal physiological function in brain and how they might relate to pathological behavior.

8. A Clinical Trial of Vasopressin in Affective Illness. Following a body of evidence indirectly linking alterations in vasopressin to cognitive and affective changes reviewed by Dr. Phil Gold, we initiated a clinical trial of the vasopressin analog DDAVP in order to study possible therapeutic effects on mood and cognition. Preliminary findings in collaboration with Drs. Phil Gold, James Ballenger, and Herb Weingartner indicate marked and statistically significant improvement in some measures of learning and memory on active drug compared to placebo, with loss of effect occurring gradually upon placebo substitution. Effects on mood have been less robust and consistent, although antidepressant effects in several patients have been noted and a marked improvement in mood and in hallucinations was observed in one patient during active drug administration and was followed by a rebound exacerbation upon placebo substitution. This ongoing clinical trial, in addition to the measurement of vasopressin following hypertonic saline administration and the measurement of vasopressin in CSF, may help clarify a possible neuroregulatory role of this peptide in some aspects of behavior and cognition.

9. Cognitive Function in Affective Illness. A series of studies in collaboration with Drs. Victor Reus, Monte Buchsbaum, Edward Silberman, and Herb Weingartner have been designed to explore the possible relationship of hemispheral laterality to affective function. Initial studies suggest that intravenous d-amphetamine produces asymmetrical cerebral hemispheral activation in man. Both hemispheres show increases in averaged evoked potentials following

amphetamine compared to placebo during tasks that are not specifically attended to; in contrast the right hemisphere appears to be selectively activated by amphetamine during relevant tasks requiring selective attention.

Depressed patients compared to both spouse and normal control populations show significant defects in abstracting ability, as measured on a Halstead Category test (in collaboration with Dr. Robert Savard). Bipolar patients perform more poorly on the test than do unipolars; older bipolar patients show the most marked deficits. The relationship of these cognitive impairments to biological parameters is currently being explored.

Preliminary findings in collaboration with Kathleen Squillace and Dr. Edward Silberman suggest that depressed patients may have lower thresholds to olfactory stimuli than manics and may experience odors in a qualitatively altered fashion.

10. Laboratory Models for Psychosis (In Collaboration with Kathleen Squillace). Since repeated psychomotor stimulant administration in man is associated with many behavioral changes bearing some resemblance to both mania and schizophrenia, we are investigating the development of behavioral pathology and associated biochemical changes during repeated cocaine administration in rats and monkeys. We have documented that several aspects of behavioral pathology, including hyperactivity, stereotypy, and, in the rhesus primate, motor inhibition show behavioral sensitization rather than tolerance with repeated application of the same dose. Preliminary data suggest that this behavioral sensitization or reverse tolerance may be associated with alterations in both pre- and postsynaptic dopamine receptor function. Cocaine pretreated animals are hyposensitive to the behavioral inhibiting effects of low doses of apomorphine (presumably acting at presynaptic receptors), while they are more responsive to high doses of apomorphine (acting at postsynaptic receptor sites).

In addition, in collaboration with Drs. Agu Pert, Candace Pert, and Jack Rosenblatt, we have observed the development of increases in α- and β-adrenergic receptor numbers following chronic cocaine administration compared to controls.

Preliminary studies also suggest that co-treatment of chronic cocaine animals with lithium carbonate blocks both the behavioral sensitization to cocaine's activating effects, as well as inhibiting the development of alpha and beta receptor alterations. Repeated indirect dopamine agonist challenges with cocaine and its associated increases in motor activity may thus be a useful partial model for the hyperactivity of mania and may help us to understand possible mechanisms of lithium's action.

In response to higher doses of cocaine or a related local anesthetic without psychomotor stimulant properties (lidocaine), animals show the development of seizures to repetition of previously subconvulsant doses. This pharmacological kindling-like effect bears some resemblance to electrophysiological kindling in which repeated stimulation of discrete areas of brain, particularly in the limbic system, eventually produces major motor seizures to the original stimulus characteristics which were nonconvulsive. Kindling appears to represent a long-lasting change in synaptic connectivity and has been studied by others as a model for learning and memory, as well as epileptogenesis. We have demonstrated, in collaboration with Dr. Agu Pert, that amygdala kindling induces long-lasting alterations in behavior, both in normal exploratory behavior and in responsivity to cocaine and lidocaine injections. The opiate peptides endorphin and enkephalin, which themselves produce seizures upon intraventricular administration, are not critical to the kindling process, as pretreatment of animals with large doses of morphine or naloxone does not affect the rate of amygdala kindling.

In collaboration with Drs. Charles Kennedy and Louis Sokoloff we have studied regional metabolic activity using the ^{14}C-2-deoxyglucose method during lidocaine-kindled seizures. Following repeated lidocaine-induced seizures, marked increases in metabolic activity are observed in a variety of limbic structures including amygdala, hippocampus, septum and entorhinal cortex. Repeated lidocaine administration not only leads to the development of seizures to a previously subconvulsant dose, but also leads to the progressive development of abnormal cognitive and eating patterns, as well as marked increases in aggressivity. These behavioral changes, taken in conjunction with the relative specificity for limbic structures as demonstrated with ^{14}C-2-deoxyglucose, suggest that repeated lidocaine administration may be a particularly useful model for the study of temporal lobe epilepsy and its associated behavioral pathology in man. We have previously highlighted that both electrophysiological and pharmacological kindling models may be useful explanatory concepts in understanding the progressive development of psychosis in some patients suffering from temporal lobe epilepsy. Similarly, this concept may be useful in conceptualizing how repeated episodes of alcohol withdrawal (which are associated with increases in neuronal excitability and may act as a kindling stimulus) are also associated with increased severity of alcohol withdrawal symptomatology and even the development of seizures and DT's following multiple withdrawal episodes.

Consistent with other reports in the literature, our clinical life-chart data in hospitalized patients reveal that in many instances manic-depressive illness is characterized by recurrences of increasing severity measured by either increasing rapidity of cycling or increasing duration of illness. These findings are also supported by a longitudinal study of chronically hospitalized untreated manic-depressive patients during the prepsychopharmacology era (in collaboration with Dr. Neal Cutler). Study of the mechanism underlying the long-lasting and progressive changes associated with behavioral sensitization to the psychomotor stimulants and kindling may be useful in conceptualizing some of the mechanisms underlying the recurrent, cyclic, and at times progressive severity of affective illness over time.

D. Proposed Course of Project:

The clinical trial of carbamazepine will continue in an effort to define the spectrum of acute and prophylactic clinical response, as well as defining clinical and biological subgroups of patients which may respond to this compound compared with lithium carbonate. Biochemical and endocrinological studies of carbamazepine will continue in order to explore its possible pharmacological effects and mechanisms of action in man. Animal studies will also be undertaken to study carbamazepine's effects on behavioral sensitization and kindling, as well as its effects on a variety of neurotransmitter receptor systems.

We will continue to study the relatively specific dopamine and noradrenergic receptor agonist compounds piribedil and clonidine. We will explore whether low doses of these compounds, which are postulated to work preferentially at presynaptic receptors, are useful in the treatment of manic illness.

In collaboration with Drs. Chris Gillin and Natraj Sitaram, a variety of studies will explore possible cholinergic alterations in affective illness based on their earlier EEG findings suggestive of cholinergic supersensitivity in depressed patients. A clinical trial of lecithin will be undertaken in an effort to treat manic patients.

A possible role in affective illness of the newly discovered endogenous peptide systems will be further explored with a variety of techniques including acute infusion of these agents themselves, blockade of endogenous opiate receptors with naloxone, and clinical trials with chronic administration of selective compounds including vasopressin. In addition we will continue to measure multiple peptide compounds in CSF in patient populations and controls in an effort to systematically document alterations in relation to psychopathology, as well as normal physiological functions of these compounds in man.

Using our population of 43 normals studied with lumbar punctures, we will explore possible biological relationships to normal variations in cognitive, motor, physiological and personality functions.

In addition to our use of the anticonvulsant carbamazepine, several areas of the interaction of seizures and psychosis will be explored in detail. In collaboration with Dr. Edward Silberman we are developing a psychosensory rating scale to document the relative occurrence of typical symptoms of temporal lobe epilepsy in manic, depressive, schizophrenic, and control populations, including patients with temporal lobe epilepsy. Behavioral and neurotransmitter mechanisms associated with cocaine, lidocaine, and electrophysiologically-induced changes in the limbic system will be further explored. Studies will continue in an attempt to better understand the clinical and laboratory effects of lithium carbonate and carbamazepine, two drugs which appear to possess both acute and prophylactic efficacy in some patients with manic-depressive illness.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 00049-05 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Psychosocial Factors in the Prognosis of Melanoma
Formerly: Psychosocial Aspects of the Progression of Malignant Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effect of various psychosocial factors on host resistance to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from others who either remain disease-free or have a delayed recurrence. At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets, and (4) melanoma adjustment self-rating score.

In a second group of 30 melanoma patients, findings (1) and (4) were replicated as predictors of relapse within one year.

PHS-6040
(Rev. 10-76)

Project Description

Objectives

This study is aimed at developing a detailed characterization of the psychosocial factors which distinguish those patients who suffer recurrence of malignancy from those who remain disease-free or who have a delayed recurrence. Data is being gathered to test several current hypotheses concerning psychosocial antecedents of the onset of physical illness and predisposing psychobiological states.

Fifty to 100 patients with "surgically-cured" Stage II Melanoma will be studied. We plan a multi-level assessment of each patient. In addition to the biological measures gathered on the patient, we will characterize the patient's genetic history, past psychosocial history, level of personality development, current life stress and present psychological state. We will accomplish this by (1) psychiatric interview, (2) objective assessment of a five minute speech sample, and (3) standardized questionnaires including MMPI, Holmes and Rahe Schedule of Recent Experience, SCL-90, Rotter Internal-External Locus of Control Scale, Beck Depression Inventory and the Gottschalk In-Out Scale.

The patients are seen shortly after their "curative" surgery and will be followed up at 2-month intervals, at which time we will obtain the Holmes-Rahe Scale, the SCL-90, Helplessness-Hopelessness Index and Beck Depression Inventory.

At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets, and (4) melanoma adjustment self-rating score. We are presently examining this finding in a third group of patients. The ABO finding did not hold up but the melanoma adjustment score did.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00132-05 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biological and psychopharmacological evaluation of schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
In the United States alone, it is estimated that 2 million people suffer from schizophrenia. Because of its very nature, this disorder is difficult to study. Pharmacological treatment is a particularly effective intervention in schizophrenia, but not a cure for all patients. Experimental and clinical findings have indicated that schizophrenia is a heterogeneous psychiatric disorder with biochemical, psychophysiological, psychological and genetic components. Patients are not constantly psychotic and their clinical state may change, regardless of medication conditions. However, we are now able to study a schizophrenic patient population intensively and longitudinally, with and without pharmacological approaches during different clinical states. We are beginning to ask fundamental questions about the nature of schizophrenia, indications for pharmacological treatment, vulnerability to psychotic decompensation and the role that genetics may play in the transmission of the disorder.

Our work integrates the multidisciplinary approaches in an effort to understand this complex disorder which may lead to a more effective treatment.

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Project Description

The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia
2. Evaluation of the dopamine, other amine and peptide hypotheses of schizophrenia
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia
5. Evaluation of novel treatments such as hemodialysis.

Methodology

One to 14 days after admission in a double-blind fashion, patients are taken off their neuroleptic medication and put on placebo. After this 2-week "washout period," patients go through a 3- to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, β-endorphin, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide, lithium, diazepam, GHB, naltrexone, muscimol or propranolol. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a 2-week placebo "buffer" period. The last of these periods is either followed by another test period or immediately followed by a 4-week clinical period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data-collection system allows us to evaluate the individual variables and drug responses against one another and to observe the patient in different clinical states. It enables us to develop psychosocial, endocrine, biochemical and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria as developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria (RDC) as described by Dr. Spitzer and his collaborators to define diagnostically our patient population. This coming year, RDC will be replaced by somewhat more descriptive DSM III criteria.

The 4-East Nursing Staff has been trained in the application of the Bunney-Hamburg psychosis, depression, and mania scale and we have added a scale to measure extrapyramidal side effects (EPS) and sedation. The Brief Psychiatric Rating Scale modified by the NIMH behavior rating scale committee under Dr. D.L. Murphy was adopted for biweekly ratings by our nursing staff and is used weekly by "blind" psychiatrist raters.

Hemodialysis of Schizophrenia

Five patients have completed a 20-week hemodialysis trial (10 real and 10 sham) in a double-blind evaluation of the potential for this experimental procedure. A special cartridge has been developed to maintain the double-blind. Patients will undergo drug-free testing, including CSF studies, endocrine and psychophysiological testing. This will provide an opportunity for collaboration with investigators in other institutes who are interested in the effects of studying hemodialysis in a nonuremic population. (Drs. Balow, Schulz)

This project stretches the expertise of psychiatrists and nursing staff alike because it requires severely ill psychotic patients to remain engaged in the project for up to 6 months without intervention of antipsychotic agents. Mrs. S. Beal and her staff are to be complimented for their collaboration and support.

Dr. Schulz presented the research design at the American College of Psychopharmacology meeting. An abstract will be published in the *Psychopharmacology Bulletin*. A paper outlining informed consent issues and management problems involved with this widely publicized procedure was submitted for publication in collaboration with Dr. Fletcher. We received over 150 requests for admission for this protocol: 15 patients were screened and 10 patients were admitted for evaluation. No results are available for publication as the blind has not been broken.

New Assays and Drugs

In collaboration with the Section on Biochemistry, BPB and the Clinical Psychobiology Branch, new assays for measuring peptides in spinal fluid have been started. We presently have drugs available that are relatively specific for the different neurotransmitter systems that we are studying.

Major Findings: Pharmacological

A. Dopamine Stimulation

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, seems a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive I.V. amphetamine and I.V. placebo during the initial test period. This is repeated during pimozide withdrawal and/or lithium.

A standardized rating system has been developed for quantitating behavioral change before, during and after the infusion interview. Additionally, blood samples are taken regularly during the day to monitor amphetamine blood levels, serum prolactin, norepinephrine and DBH levels. Clinically, schizophrenic patients appear to respond differently to amphetamine than depressed patients, although there is heterogeneity within each group. (Several papers are in preparation or have been submitted.) Strikingly, depressed patients do not become more psychotic; only 18 of 96 schizophrenic patients showed increased psychotic symptoms. However, within this schizophrenic group the more severely psychotic patients may improve, while some patients in early remission may get worse. Preinfusion spinal fluid MHPG correlated significantly with the change in psychosis. Neither chronic pimozide (neuroleptic) pretreatment nor lithium attenuated the behavioral effects of amphetamine. No increased behavioral response was observed following amphetamine administrations during the pimozide withdrawal phase (DA receptor supersensitivity). Serum norepinephrine is raised 15 minutes after the acute amphetamine administration. This rise over baseline is not affected by either pimozide or lithium. Serum DBH does not seem to be affected by amphetamine. This suggests that amphetamine affects newly synthesized, rather than stored, norepinephrine. However, there may be a competitive inhibition of DBH by amphetamine. Comparative animal data are not available. Lithium, which is supposed to prevent stimulation-induced release of norepinephrine, had no effect upon the amphetamine-induced rise in norepinephrine. A paper has been submitted for publication. Prolactin levels were increased 25 and 40 min. postamphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day--between 8-9 a.m.-- showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary.

B. Dopamine Infusion

In collaboration with Drs. Zis, Gold and Yen, Dr. DeFraites initiated DA infusions to measure prolactin, growth hormone, LH and FSH in schizophrenic patients during drug-free, neuroleptic-medicated and neuroleptic-withdrawal phases. DA does not cross the blood-brain barrier and acts, presumably, at the DA terminals of the pituitary and median eminence. Preliminary data indicate that schizophrenic patients may have an abnormal LH response and a supersensitive response to DA in the neuroleptic-withdrawal phase.

C. Dopamine Receptor Blockade and Schizophrenia

(a) Pimozide in acutely psychotic patients - Following a washout period, patients are started in a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This antipsychotic agent is of especial interest because of its relatively specific postsynaptic dopamine receptor blocking effect.

During this project endocrine studies and CSF studies are conducted. Selected patients are put on sleep study to evaluate the effects on sleep. In collaboration with Dr. Gillin we have shown that pimozide prevented amphetamine from having any effect on sleep. Rapid eye movement sleep decrease is completely prevented by pretreatment with pimozide. This is in contrast to the behavioral effects of amphetamine, which are reversed in the people who improve while drug-free after amphetamine and who then, after pimozide, become more psychotic. We also gave amphetamine infusions seven days after pimozide discontinuation to evaluate whether receptor hypersensitivity could be connected with a change in behavioral response. Data from this project are presently under analysis. However, we did not observe a strong response to amphetamine, which questions the dopamine hypersensitivity hypothesis of psychosis. A paper evaluating the effects of pimozide on sleep in psychiatric patients has been published, as well as a paper on the pimozide attenuation of the amphetamine-induced REM suppression.

Other studies include endocrine studies, cyclic AMP in CSF, lack of effect of pimozide on DBH in spinal fluid, metabolites of dopamine, norepinephrine (decrease) and serotonin in the spinal fluid and 24-hour urine collections which take place in addition to the standard battery of behavioral ratings. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay. These levels will be examined in relationship to the above-mentioned hormones and urinary free cortisol. We are now exploring CSF and endocrine variables at different time points of the pimozide trial, including during the withdrawal phase.

D. Use of Antidepressants in Schizophrenia

(a) An extensive review of the literature was conducted to ascertain the experience which has been accumulated in using tricyclic antidepressants and monoamine oxidase inhibitors in the varied types of schizophrenia. Although the number of well-controlled studies in this area is limited, it is well-documented that antidepressant medications are widely prescribed for these patients. A paper has been published in the Archives of General Psychiatry.

E. Lithium in Schizophrenia

Since the behavior activation induced by amphetamine also was attenuated by it, lithium may have some antidopamine effects. We studied lithium carbonate in psychotic and remitted schizophrenic patients: Nine of 13 psychotic patients responded with an improvement either in psychosis or depression. Diagnostic categories did not separate the responders from the nonresponders. Six of 11 remitted patients who showed postpsychotic depression improved on lithium. We are now studying biochemical and other biological variables to determine which of these may predict a response to lithium. One paper has been published and one paper submitted. Dr. van Kammen was invited to the 1978 meeting "Lithium: Controversies and Unresolved Issues," to review lithium treatment in schizophrenia and as a potential treatment of drug abuse.

F. Electrolytes and Schizophrenia

(a) We examined serum calcium (Ca) and magnesium (Mg) levels in 23 patients before and during neuroleptic treatment. Low normal Ca and Mg was an indication of emergence of EPS. Differences in Ca and Mg in drug-free schizophrenic patients were observed between diagnosis and clinical states. One paper has been published and another has been accepted for publication.

(b) Dr. Jimerson published a paper on Ca concentration in the spinal fluid related to clinical variables. He reported that CSF calcium increased when patients remitted from their psychosis.

G. Gamma-Aminobutyric Acid and the Dopamine Hypothesis of Schizophrenia

(a) A hypothesis has been developed about an ineffective feedback system regulating dopamine in psychosis. Gamma-aminobutyric acid (GABA) seems to be implicated in this role. A review paper has been published.

(b) Diazepam, one of the first compounds found to increase GABA function in the brain, was tried on our patients. We administered diazepam in doses higher than 40 mg per day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. The more psychotic the patient, the fewer the side effects there were like ataxia and sedation.

(c) GABA levels in CSF of schizophrenic patients are presently being studied in collaboration with Drs. Post and Bunney, as well as with Dr. Hare.

(d) Studies with the GABA agonist muscimol have begun.

H. Endocrinological Project

Endocrinological studies remain a major investment of the Section.

(a) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of dopamine. Prolactin, growth hormone, FSH and LH are measured in serum. Measurements thus far have been made with placebo, chronic pimozide GHB and propranolol. Increase in prolactin levels follows dopamine blockade (by pimozide) as expected. Dr. DeFraites took a major responsibility to further the Section's research involvement with this project.

(b) We also examined the growth hormone and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal, three weeks after placebo and one day after GHB withdrawal. These data are presently under analysis.

(c) Urinary-free cortisol (UFC) levels in drug-free patients, in patients on opiate, on pimozide and on the antagonist naltrexone are being examined. In contrast to the report by Drs. Chen and Carrol, schizophrenic patients do seem to have increased levels (like depressed patients). Naltrexone has no effect. Pimozide seemed to normalize UFC levels. During pimozide withdrawal a further significant decrease was observed.

(d) Chronic pimozide treatment increases serum prolactin and decreases LH. A paper has been submitted.

I. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

Baseline and probenecid accumulated metabolites of dopamine, norepinephrine, serotonin, tyramine and phenethylamine in CSF are being measured with mass spectrography.

In 12 patients HVA was evaluated twice during their stay in drug-free conditions--initially, when they were psychotic and four months later when they had improved. During their second drug-free lumbar puncture, HVA appeared to be decreased as was the psychosis.

Early evaluation of pimozide's effect upon HVA showed that eight responders had a subsequent rise of HVA but three nonresponders had a decrease. Further patient data have not supported this early observation.

Measurement of viral antibody titers in CSF has been temporarily discontinued.

CSF studies for c-AMP and c-GMP - Mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

A study of pterin cofactor activity in our patients and in normal comparison groups has started (R. Levine, W. Lovenberg and J. Ballenger). Schizophrenic patients did have similar levels to 20 controls. The variance in the schizophrenic patient was higher than in the normal controls.

CSF NE appeared to be higher in schizophrenics compared to controls and decreased relatively to improvement during pimozide treatment. Dr. Sternberg presented these data at the American Psychiatric Association meeting (May 1979).

Evaluation of peptides in CSF has been started.

Somatostatin (Drs. Gold and Roberts), Angiotensin I (Drs. Gold and Ganten) but not vasopressin (Dr. Gold) appeared to be higher in some of our patients than in normal volunteers or depressed patients.

J. Norepinephrine

(a) DBH and norepinephrine are measured in the serum of our patients while they are drug-free, on lithium and on pimozide. Neither pimozide nor lithium seemed to affect circulating serum levels of both substances.

(b) DBH in CSF of schizophrenic patients show similar levels to that of normals. Dr. Sternberg presented our data at the APA. Patients on pimozide improved in psychosis relative to the decline in CSF NE ($r = .71$, $p < .02$). A paper has been submitted. Patients with poor premorbid functioning tended to have higher DBH levels.

(c) Dr. Tallman measured β-norepinephrine receptors on leukocytes from psychotic and nonpsychotic patients during drug-free treatment and on d- and dl-high dose propranolol. There appears to be no difference between schizophrenic patients and normal controls while the receptor count stays stable over time.

(d) Dr. Marian Kafka measured α-receptor activity in our drug-free patients. Although the α-receptor activity is not different in schizophrenic patients compared to normal controls, in the male patients c-AMP production was 50% lower than in the controls. A paper was presented at the APA meeting in Atlanta, May 1978 and subsequently was accepted for publication in the American Journal of Psychiatry.

(e) A clinical evaluation of d- and dl-propranolol is under way with concomitant measurement of plasma levels, β-receptor binding, endocrines and CSF metabolites.

CSF NE appears to be elevated in some schizophrenic patients (See Section I).

K. Endorphins

(a) In collaboration with Drs. Schulz, Davis and Li we are presently giving β-endorphin infusions to schizophrenic patients for behavioral and endocrinological evaluation.

(b) A study with a longer acting opiate antagonist, naltrexone, has started. Initial results have been reported by Dr. Davis. Three out of five patients improved. Schizophrenic patients appeared pain-tolerant which is reversed by naltrexone.

Major Findings: Physiological

A. Laterality Hypothesis

The Section has continued to test the "laterality" hypothesis. This represents a novel approach to the biology of psychosis. The hypothesis is based on the demonstrated cognitive specialization in man of the cerebral hemispheres. We speculate that in psychosis there is an inappropriate intrusion of right-sided processes. To develop some relevant measures, 16 normals have been tested for differential lateralized cerebral response under a variety of stimulus conditions. These results will be compared with other clinical, biochemical and pharmacological measures.

B. CAT scan abnormalities have been found in about 60% of our patient population independent of drug use and duration of illness. Psychological and biological exploration of this finding is underway.

C. EEG Recording of Sleep

Sleep studies in relationship to amphetamine and pimozide have been undertaken. Pimozide appears to prevent the amphetamine-induced REM suppression. (See Pharmacological Findings)

D. Psychophysiological Testing

(a) Dr. Zahn of the Laboratory of Psychology and Psychopathology is testing our patients during drug-free treatment as well as during different drug conditions.

(b) Pain testing is being conducted with Drs. Davis and Buchsbaum (see Endorphins).

Major Findings: Behavioral and Psychological

A. Drug-free Improvement

We observed that eight of a group of 24 psychotic patients, whom we were not able to differentiate among clinically on admission, remitted when their antipsychotic medication was withdrawn over a 30-day period. Although these patients all had been diagnosed schizoaffective schizophrenia according to RDC classification (1975), this diagnosis did not differentiate the drug-free remitters from the schizoaffective nonremitters. The drug-free improvers appeared to have a later age of onset, a shorter time of hospitalization and better premorbid functioning. The drug-free improved male patients had low platelet MAO activity below the mean for normals, while the nonimproved patients had platelet MAO activity ranging from high to low. The drug-free improvers also responded to lower than the suggested dose ranges for the non-drug-free improvers. This continued to be present at one-year follow-up (150 vs. 484 chlorpromazine units/day). This finding may lead to a differential approach of medicating acutely psychotic patients. It suggests that if acutely schizophrenic patients do not improve on neuroleptic drugs at 4-6 weeks, the dose may need to be decreased rather than raised in some patients. The phenomenon of brief improvements during drug withdrawal will be explored further.

B. Hemodialysis

To date five patients have finished 20 dialyses each that included 10 active ones.

C. Information-processing Project

(a) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients which appeared to be shared by at least one parent in all our studied patients. A paper has been accepted for publication.

(b) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a special psychological evaluation of our patients.

Major Findings: Genetic

A. HL-A Typing Acute Schizophrenia

HL-A antigens are genetically determined human cell membrane antigens found on a variety of cell types. The genes controlling their expression are found on chromosome 6 and are thought to be linked closely to genes controlling immune responses to a number of antigens. In recent years associations of certain HL-A types and certain diseases have been found. These diseases are frequently those with associated or causal disorders of immunity, possibly the result of aberrant immune-response genes--thus their association with HL-A.

Schizophrenia may have causal or secondary immune abnormalities; therefore, a study of HL-A antigen frequencies in this disease seemed worthwhile. If found, this would constitute more evidence for the role of the immune system in schizophrenia. To date we have typed 75 schizophrenic patients.

B. Catechol-O-methyltransferase in red cells has been measured in collaboration with Dr. Gershon. Data are being analyzed.

C. Monoamine Oxidase (MAO)

Monoamine oxidase (MAO) in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating clinical and biochemical variables to activity levels. Preliminary data in our male drug-free remitted schizophenic patients indicate lower than the mean compared to a normal control population. A brief communication has been published.

Significance to Mental Health Research

These studies contribute to the understanding of schizophrenia. Our work integrates the multidisciplinary approaches to this disorder.
Pimozide attenuated the amphetamine-induced sleep changes indicating a role for dopamine in the observed sleep disorder. This finding may have implications for narcolepsy, a disorder in which amphetamine increases wakefulness.

Hemodialysis: The claims of miraculous cures (1977) are under investigation. To date (June 1979) there are many places in this country where hemodialysis is already being started as a treatment. Double-blind evaluation hopefully will tell us whether dialysis cures are placebo responses or real therapeutic effects.

Lithium may be of help in the management of schizophrenic patients, although it is less effective than neuroleptics. It may have antidepressant properties in postpsychotic depression.

Serum and CSF DBH was not lower in schizophrenic patients. CSF NE was higher than in CSF of normal controls but decreased with neuroleptic-induced improvement. These findings do not support the Stein and Weiss hypothesis of schizophrenia.

Prostaglandin-stimulated cAMP formation is less in schizophrenic males compared to normals. Does this imply that there are sex differences in schizophrenic etiology?

Schizophrenic patients as a group appeared to be more pain tolerant than normals. This could be reversed by Naltrexone, which may imply a disturbance of endorphin regulation of paramechanism in schizophrenia.

The amphetamine-induced improvement in psychosis and the relative lack of worsening during the pimozide-withdrawal-phase amphetamine infusion raises questions about the generalizability of the DA hypothesis of schizophrenia.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00081-05 BP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Heritable Characteristics of Cation Transport in Primary Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John I. Nurnberger, Jr.,M.D.	Senior Staff Fellow	BP	NIMH
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COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal area of focus for this project is the identification of heritable characteristics of ion transport which may distinguish individuals with a primary affective disorder from normal controls.

A study of sodium potassium stimulated adenosine triphosphatase (Na^+-K^+-ATPase), a cation transport enzyme, is in progress. An assay has been developed to determine the level of this membrane enzyme in bipolar and unipolar depressed patients and in normal controls. A twin study will serve to determine heritability of this enzyme.

PHS-6040
(Rev. 10-76)

Project Description

Because of negative results, the study of lithium transport in erythrocytes is now terminated.

A study of Na^+-K^+-ATPase as an indicator of cation transport is planned for summer 1979. A colorimetric assay that can detect in the range of 10-9 moles of inorganic phosphate has been developed. Enzyme activity in human erythrocytes has been determined to range from 6 to 15 nμ/min/μgm protein. This is comparable to published levels. It is planned to test the heritability of this enzyme in a group of normal twins, and then to compare normal values to those in patients with unipolar or bipolar affective disorder.

Patients will be followed with enzyme levels over time and in different mood states. It has been reported previously that this enzyme is decreased during depression.

Significance to Mental Health Research

Cation transport mechanisms depend on cell membrane characteristics that are likely to be largely genetically determined. Previous studies have demonstrated differences in cation transport mechanisms in affectively ill patients. One transport mechanism, lithium plasma/erythrocyte ratio has not been found in our studies to be correlated with lithium response or to distinguish bipolar from unipolar patients. Another mechanism, the sodium potassium stimulated ATPase, is now being investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00082-05 BP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Life Events, Family Attitudes, and Marital Dysfunction in Affective Disorders
Formerly: Life Events, Social Support and Alienation in Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the investigation of life events is to measure the relationship between the number and kind of life events, the amount of subjectively felt social support and social alienation to mood disorders in affectively disturbed patients versus controls. A new scale, the Family Attitudes Questionnaire, has been developed to measure patients' and relatives' perceptions of the morbid risk for depression or mania, of the burden these disorders create for the family, and the effect this may have on plans for marriage and child-bearing. In a study of 19 married patients/spouse couples, where marriages have been stable for at least two years, about half the spouses regret having married, but nearly none of the patients do. Less patients than spouses believe affective illness has a genetic component. Marriage and child-bearing do not appear to have been deterred by the presence of illness. The Weisman Dysfunctional Attitudes Scale was given to a pilot sample of out-patient manic depressive patients and their spouses to determine the similarity/difference in attitudes toward themselves and the world. No differences between patients or spouses were found, and they did not differ from

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Summary of Work Continued:

published values in normals. The Life Events Scale, adapted from earlier research is being used in conjunction with the family study interview of patients, their family members, and controls.

Objectives:

I. Life Events

1. To collect standardized data on life events, social support and social alienation.

2. To compare the data from a large group (approximately 100) of patients with that from matched controls.

3. To compare a) the number and kind of life events between patient and non-patient populations; b) the concentration of life events; c) the family culture associated with life events for either resident or non-resident family members in contrast to controls.

4. To correlate the number and stress of life events with the biochemical levels of DBH, COMT, MAO, etc. obtained on this same sample population.

5. To compare differences in the amount of social support perceived by patients and non-patients.

6. To compare the degree of social alienation of patients and non-patients.

II. Family Attitudes Questionnaire

1. To compare patients and relatives' attitudes toward a) the most troublesome characteristics of the acute stages of mania and depression and b) the outgoing social problems caused by the disorders and their perception of (a) the causes for the disorders; (b) the degree of risk in the general population versus relatives of patients (with mania or depression), (c) how much burden these disorders cause in comparison to various medical conditions.

2. To assess patients and relatives' attitudes toward marriage and child-bearing as an adjunct to therapeutic intervention or for genetic counseling.

3. To compare differences in attitudes among married patients, never-married patients, divorced patients.

4. To compare responses from patients with affective illness and their families to patients with schizophrenia and their families.

Objectives Continued:

III. Dysfunctional Attitude Scale

1. To collect data from patients with manic depressive illness and their spouses about their attitudes toward themselves, the future and the world.

2. To compare the differences/similarities in attitudes of patients and spouses as a test of clinical observations regarding spouse contributions to marital interaction and dysfunction.

3. To compare the similarity/difference between euthymic manic depressive out-patients and their spouses with more severely disturbed hospitalized patients and spouses.

4. To compare patients and their spouses to a matched sample of normal couples.

Method:

The Life Events Scale is a self-administered instrument completed by the proband and first degree relatives. The Family Attitudes Questionnaire is an open-ended interview asking a standard set of questions but allowing for additional comments. The Dysfunctional Attitudes Scale is self-administered.

These scales have been given to current and former NIMH patients with affective disorders and their families.

Frequency calculations, correlations, factor analysis, cluster analysis (to test relationships), and analysis of variance are used to analyze the data.

Progress to Date:

The Family Attitudes Questionnaire (FAQ) devised to quantify and assess manic depressive patients and their spouses' perceptions of the etiology, morbid risk for the disorder in relatives, and burden of the disorder to the family was extended to include schizophrenia. Analysis of the data from 19 couples (in- and outpatients, 7 BPI men, 8 women, 3 PBII men, 1 woman) reveals that 47% of the marriages post-dated the onset of illness. 53% of the well spouses felt they would not have married had they known about the illness compared to only 5% of patients ($p < .01$). Sixteen of the couples have children, 11 of whom had them after the onset of illness, although 74% of the spouses claimed they would not have children if the risk for the disorder was greater than 15% (even though in fact they estimated the risk to be that great, and in fact did have children). Seven of the 19 patients would not have been deterred from having children even if there were a 100% risk. These data indicate the defensive denial of the risk of illness on the part of both patient and spouse in their behavior, even if they may verbalize a different attitude.

Progress to Date Continued:

Awareness of this is useful in planning for genetic counseling in the major psychiatric illnesses. At this time the perceptions of other patients and their families is being obtained: additional affectively ill married patient couples, never-married patients, divorced patients. The Section on Schizophrenia Studies plans to use the FAQ to measure the perceptions of their patients and families.

A pilot study has been conducted of the difference in dysfunctional attitudes between manic depressive patients and their spouses to determine the efficacy of including families in the clinical care of patients. Thirty euthymic manic depressive outpatients and their spouses completed the Weisman Dysfunctional Attitude Scale. No significant differences were found between the patients and spouses' scores, as reported in the literature. It would appear that "Dysfunctional Attitudes" may be unrelated to affective illness in remission. One possible alternative interpretation of these findings is that the pathology of the patient is not the only contributing factor to ongoing family difficulties but that the maladaptive perceptions of their spouses do not provide corrective cognitions or reality based interpretations. To test whether such conjoint maladaptive perceptions may provide a family culture of attitudes, a matched sample of normal couples will be tested. To determine whether the euthymic state of the pilot study sample resulted in the similarity to their spouses, a sample of hospitalized patients and their spouses will be used.

Complete data has been collected on the Life Events, Social Support and Alienation Scale from 57 probands and of these from at least one of their first degree relatives. These data are being prepared for analysis to ascertain the relationship between the number and kind of life events and biochemical levels in affectively ill patients and relatives; differences or similarities between the probands and normal controls.

Proposed Course of Project:

Family attitudes will be studied in never married and divorced affective illness patients, in relatives of patients, and in schizophrenia.

Significance to Mental Health Research:

Although research on life events has already been researched for patients with a variety of diagnoses, the family culture of life events, the impact of social support or its lack, and alienation from society have not previously been related to the affective disorders. Furthermore, investigations have not previously correlated findings on life events and stress with the various biological findings in this study.

The Family Attitudes Questionnaire provides a new instrument for use in supplementing other methods of assessing patients and relatives' attitudes toward mania and depression, and the influence these may have on family planning and on response to episodes of illness.

Although only a pilot study has been completed the use of the Dysfunctional Attitudes Scale and implications from these early results raise questions about whether the scale measures depressogenic cognitions, as shown in its validation studies, or low self-esteem. The Scale has been of value in substantiating clinical observations (and offering a means of quantifying them) that maladaptation may occur in "well" family members as well as in labeled patients. A real contribution will be made if further study is able to substantiate a "family culture" of attitudes in families with an ill member as compared to "normal" families.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00083-05 BP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hypothesis of X-linkage in Bipolar and Unipolar Affective Disorder

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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0.6	0.3	0.3

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the prior annual report we described our finding of non-replication of the reported genetic linkage of X-chromosome markers (Xg blood group antigen and protan/deutan colorblindness) to Bipolar (manic depressive) illness in informative pedigrees. We have this year combined our data on color blindness linkage with that of three other collaborating centers in the Biological Psychiatry Collaborative Program of the World Health Organization (WHO). One pedigree suggested linkage, 7 pedigrees suggested non-linkage, and 8 were indeterminate. We conclude that ascertainment or diagnostic differences among centers may be causing the data to be heterogeneous, although it is possible that a relatively small proportion of Bipolar illness may be in fact linked to color blindness.

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(Rev. 10-76)

Project Description:

Objectives:

1. To develop new data pertinent to the hypothesis that some affective disorders are transmitted as a single gene on the X-chromosome. A collaborative study sponsored by the World Health Organization generated new data at three European locations.

2. To develop and test appropriate mathematical models of X-linked inheritance in psychiatric illness, and apply them to existing and newly collected data.

Methods Employed:

In a study whose results are controversial, replication by widely separated groups of investigators may be accepted as more valid than a repeat by the initial investigator. Because of the importance of the hypothesis of linkage of Bipolar manic depressive illness to the color blindness region of the X-chromosome, a collaborative study was sponsored by the Biological Psychiatry Collaborative Program of the World Health Organization. In addition to the six informative families collected at NIMH in Bethesda, eight were collected in Brussels, and one each in Copenhagen and Basel.

Attempts to standardize methodology were made by having a meeting of investigators in March of 1978 in Copenhagen, where the participants attempted to standardize diagnostic criteria and procedures. There was however no further exchange of case records, so that the reports from each center must be considered independent in regard to methodology.

Patients with Bipolar or Unipolar affective disorder and their first degree relatives (parents, sibs, children) are studied for psychiatric status, color blindness and for Xg blood type. If it is determined that a family is genetically informative for Xg or color blindness, more distant relatives are studied until a large pedigree with multiple informative sibships is obtained. Analysis is performed using the method of lod scores and using multigeneration models.

This study is distinguished by being the first to correctly analyze ascertainment through doubly ill probands (color-blind and Bipolar illness) in X-linkage, using the method of Gershon and Matthysse. A multigenerational linkage analysis is then performed using the LIPED program of Ott, with appropriate modification to take into account incomplete penetrance due to variable age of onset.

Major Findings:

Pedigree Studies:

As reported in last year's annual report, the NIMH data were clearly not compatible with close linkage. Further analysis has demonstrated that these data satisfy Morton's statistical criterion for homogeneity. The pedigree series collected in Brussels, however, was heterogeneous by this criterion. One of the eight pedigrees was clearly suggestive of linkage, and the others were ambiguous or clearly suggestive of non-linkage. The pedigrees from Basel and Copenhagen were ambiguous with regard to linkage.

Two alternate interpretations may be considered for this data. Either there is true heterogeneity in the inheritance of BP illness with a small proportion linked to red/green color blindness, or inconsistencies in methodology lead to the different findings in Bethesda and in Brussels. We favor the second possibility. The original (1974) series of reports favoring linkage suggested homogeneity of the data set, with linkage generally present. The attempts at replication in our hands were completely negative, and in the hands of the original investigator (Dr. J. Mendlewicz) yielded only one family strongly suggestive of linkage. We conclude that the initial reports were more positive than is truly warranted, and that if linkage of BP to red/green (protan/deutan) color blindness exists, it is an uncommon finding.

Proposed Course of Project:

This project is now terminated, except for additional informative families that may be identified in our ongoing analysis of a large pedigree series (see Annual Report Z01 MH 00084-05 BP).

Significance of These Findings:

The attempt to separate out an X-linked form of affective disorder is a most important and powerful investigative strategy. If the hypothesis had been confirmed, it would constitute proof of a genetic factor in the mood disorders. It would also allow more complete definition of the biological and psychological effects of an "affective disorders genotype" than has been possible until now. For these reasons, this hypothesis must be subjected to the most rigorous possible tests and methods of analysis. The collection of informative pedigrees from multiple geographic locations in different countries offered an opportunity at confirmation. The present study does not confirm or deny the hypothesis, but it does allow us to conclude that if linkage of BP to color blindness exists at all, it is present in only a small proportion of Bipolar patients.

Publications:

1. Van Eerdewegh, M. M., Gershon, E. S. and Van Eerdewegh, P.: X-Chromosome threshold models of Bipolar manic-depressive illness. J. Psychiatr. Res. In press.

2. Gershon, E. S., Targum, S. D., Matthysse, S. and Bunney, W. E., Jr.: Color blindness linkage to Bioplar illness not supported by new data. Arch. Gen. Psychiatry. In press.

3. Leckman, J. F., Gershon, E. S., McGinniss, M., Targum, S. D. and Dibble, D. D.: New data do not suggest linkage between the Xg blood group and Bipolar illness. Arch. Gen. Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00084-05 BP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Genetic-Biologic Studies of Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.0	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A family study of primary affective disorders is being performed. Data have been collected on 2535 relatives of 162 patients. Preliminary application of genetic models to the data suggested that recessive inheritance was more likely than dominant. Under this hypothesis, possible linkage of ABO blood group locus to affective illness was noted in the largest pedigree studied. Enzymes of monoamine metabolism were studied as possible genetic markers in pedigrees of affectively ill patients, but it was found that altered neither dopamine-β-hydroxylase (DBH) nor catechol-O-methyltransferase (COMT) activities were associated with transmission of affective illness within pedigrees. Extensive data analyses will be performed this year.

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Major Findings:

1. Family Study of Affective Illness

To test genetic hypotheses, we have collected psychiatric and biologic data on a series of patients with primary affective disorder, and on their relatives. By systematically examining relatives in whatever branch of the family illness was thought to exist, we have identified several pedigrees in which there is multigenerational illness, with numerous ill relatives.

One hundred sixty-two affectively ill probands remain in the study after exclusion of probands in whom further information ruled out the diagnosis of primary affective disorder. There are now 95 Bipolar I (BPI), 25 Bipolar II (BPII) and 42 Unipolar (UP) probands in the study. Two thousand five hundred thirty-five of their relatives have been diagnosed, of whom 1125 were directly examined and 1410 were diagnosed by family history information. The majority of patients have a family history of psychiatric illness (63% of UP, 73% of BPI and 69% of BPII). As in other studies, the majority of patients and affectively ill relatives are female (64% of UP patients, 60% of BPI patients and 55% of BPII patients are female).

The process of assigning final diagnosis to relatives has taken an entire year. This process requires first that two raters blindly review the interview protocol and come to a diagnosis. If they disagree, the interview is blindly referred to the senior investigator. Family history given by all persons in the pedigree about each individual is summarized. Medical records are obtained on persons who have a history of psychiatric illness that was treated. All information is then combined by an investigator responsible for the pedigree to come to a final diagnosis. At the time that this report is written, this process is nearing completion and should be complete at the end of the period covered by this report. Fifteen multigenerational families with information on 696 relatives were selected for initial genetic analysis, and all diagnostic procedures are complete on these families. Some analysis of those findings is presented below.

Based on our preliminary diagnoses, the distribution of illness in first degree relatives of the first 86 Bipolar patients is: Bipolar (I and II) 5.9%, Unipolar 10.4%, schizophrenia and other psychotic disorders 2.3%, sociopathy, drug abuse, alcoholism and sexual deviation 5.2%, cyclothymic personality 2.1%. These figures are uncorrected for age. In comparison with previous reports, these are higher than population prevalences, and comparable to our earlier studies. These figures are suggestive of a familial disorder with multiple manifestations.

The present data set will be joined with data collected by our collaborators at Yale University, headed by Dr. Myrna M. Weissman. Their data set will include 100 less severely ill (non-hospitalized) Unipolar patients, and 100 controls, with family study data. At this time, the Yale team has interviewed 47 hospitalized Unipolar patients, 85 non-hospitalized Unipolar patients, 90 normal controls, and 486 relatives. When completed during fiscal year 1980 these data plus ours will be the largest available data set for testing of genetic hypotheses about the transmission of affective disorder.

We have spent a good deal of effort standardizing the interview instruments and diagnostic procedures that are followed at the Yale and NIMH centers, including separate interviewing of persons some months apart by members of each group, and review of pedigrees and pedigree analysis procedures at each site by members of both groups. We have also exchanged personnel, in that Dr. Leckman who was formerly a clinical associate in the Section on Psychogenetics is now working with our collaborating group at Yale.

2. Genetic Analysis.

Analyses of the 15 multigenerational families whose diagnostic data is complete have been performed, using complex genetic models introduced to the Section on Psychogenetics by Dr. Lynn Goldin. These models, developed under the guidance of Dr. Robert Elston in North Carolina, are the first to simultaneously utilize all the multigenerational information in a pedigree, taking into account variable penetrance, age of onset, and linkage to genetic markers, in single major locus inheritance. Preliminary tests of single major gene hypothesis in the group of 15 families has shown recessive inheritance to be more likely than dominant, but both hypotheses were rejected. The "environmental" hypothesis could not be rejected. Maximum likelihood analysis (LOD scores) for linkage under autosomal recessive hypothesis of transmission of illness was calculated for 20 markers in 10 families. No consistantly positive LOD scores were found but there is a suggestion of linkage (maximum LOD score = 1.03 at $\theta = 0$) at the ABO blood group locus in the largest family in our sample (42 individuals with known phenotypes). Further application of genetic models to maximize likelihood of linkage in this large pedigree will be performed, with the results than tested in other pedigrees in the data set, as these become available.

In attempts to resolve the heterogeneity of affective illness in our sample, the genetic and environmental hypothesis will be tested in smaller subgroups of families on the basis of: age of onset of proband, low platelet monoamine oxidase activity and other biologic characteristics in the proband, including low baseline cerebrospinal fluid 5-HIAA in the proband.

3. Identification of Genetic Vulnerability Factors in Affective Disorders.

A. Dopamine-β-hydroxylase (DBH) and erythrocyte catechol-O-methyltransferase (COMT). Distinct alleles for reduced activity of plasma DBH and erythrocyte COMT have recently been identified. We have studied whether these alleles are associated with affective illness. For COMT we have studied 62 Bipolar I, 24 Bipolar II, 33 Unipolar, 53 controls, and 607 relatives of patients. No significant differences between patients and controls were found. No association between the allele for decreased COMT activity and the transmission in illness in pedigrees of relatives was found. The sex effect (males having greater activity than females) noted in previous reports was present.

DBH: 67 Bipolar I, 21 Bipolar II, 26 Unipolar, 124 normal controls and 536 relatives were studied. No differences between patients and controls were present. Only 9 of 114 patients with primary affective disorder had the allele for very low or absent DBH activity. No relation between illness and absent DBH activity was found in their relatives, although the number of people in these families was small.

4. Other Studies.

The pharmacogenetic approach to searching for genetic vulnerability markers has been applied with the cholineric muscarinic agonist arecoline, and the adrenergic indirect agonist d-amphetamine, as described in a separate annual report (Z01 MH 00085-03 BP). Other studies of fibroblasts, enzyme activity, and receptors on leucocytes are described in annual report Z01 MN 00086-03 BP).

Future Course of Project:

Further genetic modeling will be applied to find a most likely mode of transmission of illness within the entire series of pedigrees. Models of multifactorial transmisssion and mixed models of single locus and multifactorial transmission will be applied.

Additional genetic markers have still to be analyzed, and stored platelet and plasma material from the pedigrees will be used for new markers that become available.

Further studies of childhood in the children of patients with Bipolar affective illness and personality variables in patients and relatives are underway.

Publications:

1. Leckman, J. F. and Gershon, E. S.: Autosomal models of sex effect in Bipolar-related major affective illness. *J. Psychiatr. Res.* 13(4): 237-264, 1977.

2. Reider, R. O. and Gershon, E. S.: Genetic strategies in biological psychiatry. *Arch. Gen. Psychiatry* 35: 866-873, 1978.

3. Gershon, E. S. and Liebowitz, J. H.: Affective Illness. In Goodman, R. M. and Motulsky, A. G. (Eds.): *Genetic Diseases Among Ashkenazi Jews.* New York, Raven Press, pp. 343-352, 1979.

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7. Sparkes, R. S., Targum, S. D., Gershon, E. S., Sensabaugh, G. F., Sparkes, M. C. and Crist, M.: Evidence for a null allele at the esterase D (EC 3.1.1.1) locus. Hum. Genet. 46: 319-323, 1979.

8. Major, L. F., Murphy, D. L., Gershon, E. S. and Brown, G. L.: The role of plasma amine oxidase, platelet monoamine oxidase, and red cell catechol-O-methyl transferase in severe behavioral reactions to disulfiram. Am. J. Psychiatry 136: 679-684, 1979.

9. Leckman, J. F., Gershon, E. S., McGinniss, M., Targum, S. D. and Dibble, E. D.: New data do not suggest linkage between the Xg blood group and Bipolar illness. Arch. Gen. Psychiatry. In press.

10. Van Eerdewegh, M. M., Gershon, E. S. and Van Eerdewegh, P.: X-Chromosome threshold models of Bipolar manic-depressive illness. J. Psychiatr. Res. In press.

11. Mazure, C. and Gershon, E. S.: Blindness and reliability in lifetime psychiatric diagnosis. Arch. Gen. Psychiatry. 36: 521-525, 1979.

12. Gershon, E. S., Targum, S. D., Matthysse, S. and Bunney, W. E., Jr.: Color blindness linkage to Bipolar illness not supported by new data. Arch. Gen. Psychiatry. In press.

13. Targum, S. D., Gershon, E. S., Shen, R-S. and Abell, C. W.: Screening for PKU heterozygosity in Bipolar affectively ill patients. Biol. Psychiatry. In press.

14. Targum, S. D., Gershon, E. S., Van Eerdewegh, M. and Rogentine, N.: Human leukocyte antigen (HLA) system not closely linked to or associated with Bipolar manic-depressive illness. Biol. Psychiatry. In press.

15. Gershon, E. S. and Reider, R. O.: Are mania and schizophrenia genetically distinct? In: Mania: An Evolving Concept, R. H. Belmaker and H. M. van Praag (Eds.), Spectrum Publications, Inc., Holliswood, N.Y. In press.

16. Targum, S. D. and Gershon, E. S.: Genetic counseling for affective illness. In: Belmaker, R. H. and van Praag, H. M.: Mania: An Evolving Concept. Spectrum Publications, Inc., Holliswood, New York. In press.

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PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacogenetics of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. Guroff, B.A.	Research Assistant	BP	NIMH
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LAB/BRANCH
Biological Psychiatry Branch

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Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inheritance of variability in the behavioral and physiologic effects of d-amphetamine is being studied. A small dose of amphetamine (0.3 mgm/kg) is administered to normal volunteer twins after medical and psychiatric screening. Preliminary data suggests stable and heritable responses in 1) behavioral ratings and 2) pharmacokinetics of amphetamine. An increase in serum prolactin is found in some individuals, and appears heritable. Pulse and blood pressure and subjective assessment of mood by a self-rating scale were found to be stable in individuals but not heritable. Nocturnal motor activity seems to be heritable but not stable in an individual. A twin study of rapid eye movement (REM) sleep induction response to Arecoline is also underway.

PHS-6040
(Rev. 10-76)

Other Investigators Continued:

J. C. Gillin, M.D.	Chief, Unit on Sleep Studies BP NIMH

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken, although there have been enough clinical reports of patients and relatives responding to the same medication to suggest that further study will be fruitful.

Our first pharmacogenetic protocol is a study of d-amphetamine. Individual differences in response to amphetamine have been used as a predictor of clinical response to tricyclic antidepressants and as such may be an important clue to the genetic vulnerability to affective disorders. In this project, monozygotic and dizygotic twin volunteers are being studied. All persons with pre-existing psychiatric or medical disorders that are not minor are excluded.

d-Amphetamine is an indirect agonist of dopamine and norepinephrine neurons in the central nervous system. There has been much recent interest in the effects of amphetamine and amphetamine-like compounds such as methylphenidate in mania, schizophrenia and depression.

Amphetamine may produce mood changes of excitation or depression. Results on thirteen monozygotic twin pairs and three dizygotic twin pairs show that these responses are likely to be heritable (intraclass correlation for the excitation response was 0.70 and for the depression response was 0.58). The excitation response was found to be more reproducible within an individual than the depressive response. Plasma amphetamine half-lives were also found to be reproducible in an individual and heritable. The behavioral responses were not correlated with amphetamine levels, however, suggesting heritable differences in drug response as well as drug metabolism.

Use of the patient activity monitor (PAM) indicated that the amount of nocturnal activity following amphetamine was a heritable quality ($p < .05$), but this was not found to be stable in individuals over time.

Serum prolactin was found to show wide individual differences in response to amphetamine. These responses also seemed to be stable and heritable; the normal individuals we studied had less of an increase in prolactin than schizophrenic patients studied before in another NIMH study. Data on growth hormone, cortisol, luteinizing hormone and follicle-stimulating hormone is presently being analyzed.

Averaged evoked responses (AER) have also been measured following amphetamine. One component, the augmentation-reduction measurement has been analyzed and found not to show a heritable response pattern. Other components will be analyzed.

Pulse and blood pressure responses were found to be reproducible within individuals but not heritable.

The Addiction Research Center Inventory (ARCI) scale "B" was administered to all subjects in the study. An amphetamine effect on scale scores was confirmed and the response was found to be reproducible but not heritable.

A behavioral rating system with high interrater reliability has been developed. The system is illustrated with an audio-visual teaching tape. Tapes of the infusions are being retained for research purposes. A systematic interview technique for pharmacologic studies was developed.

We are also investigating heritability of the REM sleep induction response to arecoline, a cholinergic agonist. This response has been found to differentiate affectively ill patients in the well state from controls. We have tested three pairs of monozygotic twins at this point; data are being analyzed.

Proposed Course of Project:

We plan to compare results of this study with results in a similar on-going study on patients. If the technique seems promising for differentiating people at risk for affective disorder, we proposed to investigate amphetamine response in relatives of clinic patients (protocol amendment to be written). We propose to test five more pairs of monozygotic twins in the arecoline study.

Significance:

We have noted heritable differences in response to amphetamine in behavior, pharmacokinetics, and serum prolactin level. If these responses differentiate patients with affective illness from controls, they may be of use in tracing vulnerability to mood disorder through families, and in genetic counseling of family members. The arecoline response has been shown to differentiate patients from controls.

Our behavioral rating system and interview technique will be helpful in standardizing results of studies in behavioral pharmacology.

Publications:

1. Gershon, E. S., Nurnberger, J. I., Sitaram, N. and Gillin, C.: Pharmacogenetics and the pharmacologic challenge strategy in clinical research. Prog. Neuro-Psychopharmacol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00086-03 BP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BP	NIMH
OTHER:	J. Nurnberger, M.D.	Senior Staff Fellow	BP	NIMH
	N. Sitaram, M.D.	Research Psychiatrist	BP	NIMH
	J. C. Gillin, M.D.	Chief, Unit on Sleep Studies	BP	NIMH
	E. D. Dibble, D.S.W.	Psychiatric Social Worker	BP	NIMH
	M. Kafka, Ph.D.	Physiologist	BP	NIMH
	J. Tallman, Ph.D.	Biologist	BP	NIMH
	S. Simmons, M.S.N.	Clinical Nurse Expert	CC	NURS
	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP	NIMH
	W. E. Bunney, Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Clinical Center Nursing Department, NIH; Yale University

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient clinic provides a "well state" patient population for genetic and pharmacologic studies of persons with Unipolar (major depressive) and Bipolar (manic-depressive) disorders. Such a "well state" population is crucial in identifying biochemical, pharamcologic, or physiologic abnormalities which are present in persons vulnerable to affective disorder, and which may serve as genetic markers to affective illness. As a comparison group for such studies, we have developed a carefully screened panel of monozygotic and dizygotic twins without evidence of medical or psychiatric disorder. Continuing our collaboration with the Unit on Sleep Studies, we find clear evidence of greater sensitivity to induction of rapid eye movement (REM) sleep by a cholinergic agonist (Arecoline) in well state Bipolar patients off all medications, as compared with controls. Twin studies of genetic control of this response are underway. In other studies, α-adrenoceptors on platelets were found not to differentiate between patients and normal controls. β-adrenoceptors on lymphocytes did not show correlation between members of twin pairs, suggesting nongenetic sources of variation in receptor binding. A cell-culture

Summary of Work Continued:

bank of fibroblasts taken from Bipolar patients and normal controls has been developed. First results show no difference in Type A monoamine oxidase (MAO) activity between patients and normal controls.

Project Description:

The purpose of the project is to have a "well state" patient population who would be available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.

2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.

3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.

Patients are admitted to the study upon referral from the wards of the NIMH intramural program or from various community referral resources. Sixty-five patients (33 Bipolar I, 14 Bipolar II, 16 Unipolar, 2 others), are currently enrolled in the BPB portion of the outpatient clinic.

As a control group for testing genetic variation in the normal population, we have developed a panel of 15 pairs of monozygotic twin normal volunteers. Volunteers from this panel participated in Arecoline, d-amphetamine, α- and β-adrenoceptor and fibroblast studies described in this report.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

Pharmacologic studies: Arecoline is a muscarinic cholinergic agonist which induces rapid eye movement (REM) sleep when given in sufficient dosage. This effect is blocked by scopolamine, which suggests that the effect is produced by stimulation of a central cholinergic receptor. In collaboration with the Sleep Studies Unit of the Biological Psychiatry Branch (Z01 MH 00085-03 BP), we have given low doses of Arecoline intravenously during non-REM sleep to 11 Bipolar patients who were euthymic and off medications.

Eleven patients and 13 normal controls have been studied. Arecoline (0.5 or 1.0 mg) or placebo is administered intravenously at 25 minutes past the end of the first REM period. Under placebo conditions there is no significant difference in the time to next REM period between patients (52 ± 6 minutes) and controls (63 ± 9). Both doses of Arecoline produced rapid onset of REM in patients but not in normal controls. Maximum differentiation was produced by the lower (0.5 mg) dose of Arecoline (time to next REM period: patients 11 ± 2 minutes, controls 53 ± 10). We are currently studying whether this finding may be related to previous lithium administration, and whether there is heritable variation in susceptibility to Arecoline in normal volunteer monozygotic twins. If these studies support our interpretation that the patient-control difference is due to genetic difference in sensitivity of cholinergic response to Arecoline (as opposed to effects of environment, including differences in exposure to psychotropic agents), we will proceed to study ill and well relatives of patients. These pharmacogenetic studies would test whether Arecoline response is a genetic marker of vulnerability to affective illness.

Studies of genetic variation in response to acute intravenous injection of d-amphetamine are described in a separate annual report (see project report Z01 MH 00085-03 BP). In normal monozygotic and dizygotic twin volunteers, there is genetically controlled variation in behavioral and neuroendocrine response to d-amphetamine. We plan to study outpatient clinic patients who are euthymic when off medication, to see if they differ from these normal controls. The same experimental paradigm will be followed as in the Arecoline studies, of looking for patient/control differences in a genetically determined trait, and then tracing the finding through pedigrees of patients.

Cell culture offers a way of preserving living cells with all the genes of the donor. We are developing a fibroblast "bank" from skin biopsies taken from BP patients and normal volunteer twins, in the laboratory of Dr. X. O. Breakefield of Yale University. Initial results on type A monoamine oxidase (MAO) activity (which can be measured in fibroblasts but not in platelets) demonstrated very positive correlation among members of 3 twin pairs, supporting the validity of this technique in studying quantitative variation in enzyme activity. No difference was found in type A MAO activity among eight Bipolar patients and the normal volunteer twins, and all values corresponded to the normal control values in Dr. Breakefield's laboratory.

Other biological studies: α-adrenoceptor binding was studied in the platelets of eight BP patients versus three pairs of monozygotic normal volunteer twin controls, in the laboratory of Dr. M. Kafka. No suggestive differences were found. β-adrenoceptor binding in lymphocytes in three pairs of normal volunteer twins was studied in the laboratory of Dr. J. Tallman. No significant correlation between members of a twin pair was noted.

Biological markers of a genetically transmitted predisposition to affective disorders. Linkage to known chromosomal markers is a strategy for demonstrating that a characteristic is inherited and for demonstrating the mode of inheritance. Patients and relatives in this clinic are studied for chromosomal linkage markers, such as blood group phenotype, HLA phenotype and known biochemical polymorphisms, as described in project report #Z01 MH 00084-04 PB.

Clinical studies of the well state in affective disorders: Pre-existing conceptions on familial and genetic questions in affective illness, and expectations of affective illness and its consequences in one's self and relatives, are being studied through questionnaire and clinical interviews. This information may prove useful for genetic counseling in psychiatry, a service for which there is a growing demand. A controlled study of marital adjustment using the KDS-15 questionnaire developed by E. Frank (Univ. of Pittsburgh) has been performed in 15 couples with Bipolar illness in one member, to continue studies of the disordered marital relationships that characterize Bipolar affective illness. Results are not yet ready for presentation.

Significance of the Study:

Identification of a biologic vulnerability detectable in the well state in Bipolar manic depressive illness would be of great importance for clinical treatment and also for identifying persons who might become ill in the future, including relatives of patients.

Proposed Course of Project:

Studies of CNS and peripheral sensitivity to agonists and antagonists of monoamine and cholinergic receptors will continue to have highest priority over the coming years.

Publications:

1. Gershon, E. S. and Hamovit, J. R.: Genetic methods and preventive psychiatry. Prog. Neuro-Psychopharmacol. In press.

2. Nurnberger, J. I., Gershon, E. S., Murphy, D. L., Buchsbaum, M.S., Goodwin, F. K., Post, R. M., Lake, C. R., Guroff, J. J. and McGinniss, M. H.: Biological and clinical predictors of lithium response in depression. In: Gershon, E. S., Kline, N. S. and Schou, M. (Eds.): International Lithium Conferences: Controversies and Unresolved Issues. Excerpta Medica, ICS Series, New Jersey. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00116-05 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Interaction of Neurotransmitters and Membrane Proteins at Bimolecular Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
Other:	Robert Blumenthal	Visiting Fellow	LTB NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been completed.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00117-04 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

α- and β-Adrenergic and Prostaglandin Receptors in Human Blood Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
	John F. Tallman	Biologist	BP NIMH
Other:	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)
Section on Psychobiology, BPB, NIMH; Section on Neuropsychopharmacology, BPB, NIMH; Laboratory of Clinical Psychopharmacology, NIMH; Clinical Psychobiology Branch, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha- and β-adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic drugs to activate or inhibit adenylate cyclase is used as a measure of their biological function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on the blood cells is being measured. These correlative measures are used to assess α- and β-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

Objectives:

(1) To develop methods to study the physiological function of α- and β-adrenergic receptors in easily obtainable human tissue. (2) To study alterations in receptor number or function in pathological conditions which are characterized by altered receptor sensitivity and in psychiatric diseases. (3) To provide an experimental model for the study of drug and/or physiologically induced changes in central receptor sensitivity in man. (4) To understand the membrane perturbations or alterations connecting receptors occupied by their agonists with the inhibition or activation of adenylate cyclases and subsequent physiological events.

Methods Employed:

To measure α-adrenergic receptors, human platelets are prepared from freshly drawn blood. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α-adrenergic antagonist binding. To measure β-adrenergic receptors a leukocyte preparation is made from human blood using a dextran polymer to induce rapid settling of the erythrocytes. The leukocyte-rich plasma is centrifuged at low speed to yield a leukocyte pellet. This pellet is washed several times in 0.9% saline solution and is used to measure binding of a tritiated β-antagonist and production of cAMP.

Cyclic nucleotides are determined after incubation of intact leukocytes or platelets with the drugs under study by a competitive protein binding assay for cAMP or by radioimmunoassay for cGMP.

Major Findings:

(1) Platelets from adult male control subjects have about 1.5 times the number of α-adrenergic receptors and produce about 1.8 times as much cyclic AMP when stimulated with prostaglandin E_1 as platelets from adult female control subjects.

(2) Alpha-adrenergic receptor function is normal in platelets from a group of chronic schizophrenic patients.

(3) Cyclic AMP production is decreased in platelets from a group of male schizophrenic patients.

(4) Platelets from female patients with idiopathic hypertension have about 1.5 times as many α-adrenergic receptors as platelets from female normotensive control subjects. Platelets from male hypertensive patients produce about one-half as much cAMP as those from normotensive male control subjects.

(5) Beta-adrenergic receptor function in leukocytes from hypertensive patients of both sexes is similar to that in leukocytes from control subjects.

Significance to Biomedical Research and the Program of the Institute:

It is anticipated that results obtained from these experiments will be directly applicable to an assessment of receptor functioning in various human disease states and in monitoring the physiological effects of various drug treatments. If the peripheral α- and β-adrenergic receptors are related to or can serve as a model for central nervous system adrenergic receptors, valuable information may be obtained for the first time about the functioning of these central receptors in psychiatric diseases.

It is possible that adrenergic receptor function in the central and peripheral nervous systems is similar to that measured in platelets and leukocytes. A group of schizophrenic patients showed no abnormality in α-adrenergic function. Male schizophrenic patients, however, showed diminished cAMP production which, if present in central nervous system neurons, might lead to decreased phosphorylation of, and activation of, some cell proteins resulting in the deficits that characterize schizophrenia. Idiopathic hypertension may be a disease of stress which involves central sympathetic adrenergic nervous system function as well as adrenergic control of arteriolar smooth muscle constriction. An increased number of α-receptors may mediate hypertension peripherally in women. A decrease in stimulated cAMP production in male patients may increase blood pressure peripherally via arteriolar smooth muscle constriction as has been shown in animal studies, and a decrease cAMP production in central inhibitory centers may increase blood pressure by removal of inhibition of vasoconstriction. Beta-adrenergic receptor function, at least that reflected in leukocytes, does not seem to be altered.

Proposed Course:

Examination of the mechanism of information transfer from α- and β-adrenergic receptors to adenylate cyclase is continuing. Studies of these receptors in patients with hypotension, affective illness, and the α-receptor in hyperactive children are underway as well as further studies of α-receptor function in schizophrenia.

Publications:

Kafka, M.K., van Kammen, D.P., and Bunney, W.E. Jr.: Reduced cyclic AMP production in the blood platelets from schizophrenic patients. Amer. J. Psychiat. 136: 685-687, 1979.

Kafka, M.S., Lake, C.R., Gullner, H.G., Tallman, J.F., Bartter, F.C., and Fujita, T.: Adrenergic receptor function is different in male and female patients with essential hypertension. Clinical and Experimental Hypertension, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00118-03 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Induction of β-Adrenergic Receptors in HeLa Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Research Biologist	BP	NIMH
Other:	Richard C. Henneberry	Senior Scientist	LMB	NINCDS
	Craig C. Smith	Chemist	BP	NIMH
	Ayub Yousufi	Visiting Fellow	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

β-Adrenergic receptors are present in HeLa cells and the number of these receptors can be increased by culturing the cells in the presence of butyrate. At low concentrations of butyrate, receptor number is increased and at higher concentrations, both number and ability to activate adenylate cyclase are enhanced. The β-adrenergic receptor in HeLa can also directly enhance phospholipid methylation, and this effect is not mediated by activation of adenylate cyclase. The amount of phosphatidylcholine in the receptor's microenvironment affects the apparent number of β-adrenergic receptors.

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(Rev. 10-76)

Cultured HeLa cells contain binding sites which fit the classification as β-adrenergic receptors. A radioactive β-adrenergic antagonist, 1-[^{3}H]dihydroalprenolol, binds to the cells in a rapid, reversible and stereospecific manner consistent with its pharmacological properties. The binding of 1-[^{3}H]-dihydroalprenolol is of high affinity, and occupancy of this high affinity binding site can be correlated with the activation of adenylate cyclase by known β-adrenergic agonists.

It is possible to induce a number of biochemical changes in HeLa cells by including short-chain, saturated fatty acids in the culture medium. The most effective fatty acid is butyrate, and it is capable of inducing G_{M3} ganglioside synthesis, sialyl-transferase, alkaline phosphatase, α-chain of human-chlorionic gonadotropin, and a particular elongated shape. Additionally, the number, but not affinity, of the 1-[^{3}H]dihydroalprenolol binding sites is increased after treating the cells for 24 h with either low (0.5 mM) or high (5 mM) sodium butyrate. Along with the increased number of binding sites, there is an increase in the ability of these sites to activate adenylate cyclase in the presence of the higher butyrate concentration.

Thus, the increase in the number of β-adrenergic binding sites can be separated from the increased "coupling" to adenylate cyclase, since increased receptor number is obtained by culturing the cells at a low butyrate concentration (<1 mM) while higher concentrations of butyrate are required to obtain "coupling" of receptor and adenylate cyclase. Thus, an increased number of receptors can be obtained by preincubating at low butyrate concentrations (0.5 mM); shifting the medium to one containing a higher (5 mM) butyrate concentration results in an efficient coupling to adenylate cyclase. Using this paradigm, a number of factors affecting coupling of receptor and adenylate cyclase have been studied.

Butyrate-treated HeLa cells have also proven a valuable tool in studies on the involvement of phospholipid methylation in β-adrenergic receptor function. Two asymmetrically distributed membrane-bound enzymes are present in many tissues including reticulocytes and HeLa cells which synthesize phosphatidylcholine by successive N-terminal methylations of phosphatidylethanolamine. The first enzyme, methyl transferase I, located on the cytoplasmic side of the plasma membrane, requires Mg^{+2}, has a high affinity for the methyl donor S-adenosyl-L-methionine, and catalyzes the formation of phosphatidyl-N-monomethylethylethanolamine. Methyltransferase II, located on the external aspect of the plasma membrane, adds two additional methyl groups to phosphatidyl-N-monomethylethylethanolamine to form phosphatidylcholine. It has a lower affinity for S-adenosyl-L-methionine and does not require Mg^{+2}. By these sequential reactions, phosphatidyl ethanolamine, methylated to phosphatidylcholine, can be translocated to the external side of the plasma membrane. This phenomenon may account for the asymmetric distribution of phospholipids in many cells.

It has been shown in intact rat reticulocyte ghosts that activation of β-adrenergic receptors causes activation of both methyltransferases leading to the translocation of phospholipids from the cytoplasmc side of the membrane to the outside. In view of the potential importance of this phenomenon to β-adrenergic receptor function, a series of similar experiments were also carried out in HeLa cells.

In HeLa cells the incorporation of methyl groups into the phospholipid fraction is increased in intact butyrate-treated cells in the presence of β-adrenergic agonists. In order of potency of these compounds (l-isoproterenol > l-epinephrine > l-norepinephrine > d-isoproterenol) is consistent with the ability of these compounds to activate adenylate cyclase or cAMP production in intact cells. Activation of methyl group incorporation by the β-adrenergic receptor is extremely rapid, and can be correlated with the receptor occupancy; β-adrenergic antagonists such as l-alprenolol and propranolol block methyl incorporation into the phospholipids, while α-adrenergic antagonists like phentolamine are inactive as antagonists. It should be noted that the increase in methylation is due to receptor occupancy rather than cAMP production because cholera toxin under conditions which will maximally activate adenylate cyclase has no effect on phospholipid methylation.

There is an active system for the turnover of the pool of phosphatidylcholine synthesized in response to β-adrenergic receptor activation. One of the major products in the degradative scheme is a lysolecithin, the product of phospholipase A action on lecithin. This phospholipase is a Ca^{+2} requiring enzyme present on the cell surface, and apparently it is also activated in the presence of β-adrenergic agonists. It is not known whether cAMP is required for this activation. Additional products such as free choline have also been observed indicating that additional degradative steps may also be important. Thus, the half-life of the newly synthesized phosphatidylcholine is quite short--also consistent with its involvement in β-adrenergic function.

The apparent number of β-adrenergic receptors may also be partly under phospholipid control. Incubation of resealed rat reticulocyte ghosts with S-adenosyl-L-methionine resulted in an increase in β-adrenergic receptor number. This increase seemed to be due to the unmasking of cryptic receptors by changes in the microenvironment and was blocked by including a high concentration of S-adenosyl-L-homocysteine (a competitive inhibitor of methylation) along with the S-adenosyl-L-methionine. Several compounds (N^6-methyladenosine and 4-deazaadenosine) will inhibit the hydrolysis of S-adenosyl-L-homocysteine in various cells and tissue resulting in an increase in the ratio of S-adenosyl-L-homocysteine. When butyrate-treated HeLa cells were cultured in the presence of these compounds, phospholipid N-methylation was specifically inhibited (carboxymethylation and RNA methylation were unchanged). Concurrent with inhibition of N-methylation, there was a reversible decrease in the number of β-adrenergic receptors and in l-isoproterenol-activated cAMP production. Thus, methylation of phospholipids affects the exposure of the β-adrenergic receptor on the HeLa cell surface and modulates the ability of this receptor to activate adenylate cyclase.

A role of methylation in the modulation of receptor sensitivity is suggested by these studies of phospholipid metabolism. One hypothesis under investigation is that although there is an immediate increased synthesis of phosphorytidylcholine after receptor occupancy by agonists, the long term net result is depletion of a critical pool of phosphatidylcholine in the microenvironment of the β-adrenergic receptor, leading to receptor desensitization. Recovery of sensitivity may depend on resynthesis of phosphatidylcholine or translocation of the receptor to a new lipid environment.

Significance to Biomedical Research and the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of in vivo effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states.

Proposed Course:

Research is currently underway into the following questions:

1. What is the mechanism of catechoalmine-induced subsensitivity?

2. What is the nature of the second event which occurs after treatment of the HeLa cells with high concentrations of butyrate: Is there a membrane-bound factor whose synthesis is necessary for efficient coupling to occur?

3. Can we develop receptor- or butyrate-resistant mutants?

4. What is the role of membrane fluidity in these processes?

Publications:

Tallman, J.F., Smith, C.C., and Henneberry, R.C.: Properties of β-adrenergic receptors in untreated and butyrate-treated HeLa cells. Biochim. Biophys. Acta, 541: 288-300, 1978.

Tallman, J.F., Henneberry, R.C., Hirata, F., and Axelrod, J.: Control of β-Adrenergic Receptors in HeLa Cells. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. Oxford, Pergamon Press, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00119-03 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Putative Neurotransmitters and Modulators in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Biologist	BP NIMH
Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Pauline Lerner	Chemist	BP NIMH
	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.4	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methods were developed for the determination of cAMP, cGMP, γ-aminobutyric acid (GABA), substance P, endogenous opiate ligands and cortisol. Using these methods, it is possible to measure these compounds in a variety of tissues and under many physiological conditions. In particular, all of the above compounds may be measured in cerebrospinal fluid and plasma from human subjects. With the available cerebrospinal fluid from patients under study in the Sections of Psychobiology and Neuropsychopharmacology, no differences between patients with schizophrenia and affective disorders have yet been demonstrated for cAMP, cGMP and GABA.

PHS-6040
(Rev. 10-76)

Using radioimmuno, radioreceptor, or radioenzymatic methods it was possible to develop assays for cAMP, cGMP, GABA, substance P and endogenous opiate ligands. cAMP was measured by a protein binding method which utilized protein kinase from bovine adrenal cortex. The cAMP in the sample to be determined competes with radioactive cAMP for a limited number of binding sites on protein kinase. By using varying amounts of unlabeled cAMP, it is possible to construct a standard curve and thus quantitate the amount of cAMP in the sample. Similarly, GABA and endogenous opiate levels were determined by measuring the ability of the sample (or known amount of GABA or endogenous opiate) to displace the radioactive ligand from a brain membrane preparation which binds to GABA or opiates specifically and with a high affinity. Substance P and cGMP were measured using a radioimmunoassay with antibodies either prepared by us or purchased.

We have put a large effort into developing antibodies against β-endorphin to use the antibodies in developing an assay for β-endorphin which can be used on clinical samples. This assay is good enough to detect the endogenous levels of this compound in body fluids such as plasma or CSF. However, reproducibility remains something of a problem. Efforts will continue to improve this antibody and extraction procedure until a successful assay is obtained. At present our assay is capable of determining the levels of β-endorphin after infusions.

Significance to Biomedical Research and the Program of the Institute:

Measurement of transmitters and modulators of brain function in cerebrospinal fluid may be important for understanding the metabolism and turnover of these compounds in brain. If differences exist between groups of patients, it may be possible to implicate a transmitter or biochemical event as being important in affective disorders or schizophrenia.

Proposed Course:

Samples are being collected to measure β-endorphin in control states and in patients with different diseases. Assays for cyclic nucleotides are being carried out on a routine basis. An assay for CSF cortisol is presently being developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00120-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

GABA-Benzodiazepine Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dorothy W. Gallager	Senior Staff Fellow	BP NIMH
	John F. Tallman	Biologist	BP NIMH
Other:	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project Z01 MH 00157-01 BP.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00121-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Dopamine-β-hydroxylase in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP	NIMH
Other:	James C. Ballenger	Clinical Associate	BP	NIMH
	L. Frank Major	Clinical Associate	CN	NIMH
	C. Raymond Lake	Clinical Associate	LCS	NIMH
	Robert M. Post	Chief, Section on Psychobiology	BP	NIMH
	Frederick K. Goodwin	Chief	CP	NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, NIMH; Laboratory of Clinical Science, NIMH
Clinical Psychobiology Branch, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine-β-hydroxylase has been measured in cerebrospinal fluid and plasma from humans and laboratory animals treated with various drugs. Disulfiram treatment of humans caused no significant change in dopamine-β-hydroxylase in cerebrospinal fluid or plasma. Administration of disulfiram to monkeys caused no change in cerebrospinal fluid dopamine-β-hydroxylase, but induced a large, persistent increase in plasma dopamine-β-hydroxylase. Chronic treatment of cats with monoamine oxidase inhibitors caused a significant decrease in cerebrospinal fluid dopamine-β-hydroxylase. The monoamine oxidase inhibitors did not change brain dopamine-β-hydroxylase levels. Treatment of cats with phenoxybenzamine or clonidine caused cerebrospinal fluid dopamine-β-hydroxylase to change in the predicted directions based on their α-antagonist and α-agonist properties, respectively.

PHS-6040
(Rev. 10-76)

Objectives:

Dopamine-β-hydroxylase (DBH) and norepinephrine are released from storage vesicles when noradrenergic neurons fire. DBH is being measured in cerebrospinal fluid (CSF) as a possible indicator of central noradrenergic activity. The differential effects of drugs on DBH in CSF and in plasma are also being studied.

Methods:

A previously reported radioenzymatic assay for DBH has been modified to achieve the necessary sensitivity to measure reliably the very low DBH activity in CSF.

Major Findings:

Disulfiram, a drug used on alcoholics because it inhibits aldehyde dehydrogenase, also inhibits DBH in situ. Since it is a reversible inhibitor of DBH, it does not inhibit the DBH released into CSF or plasma. In humans, disulfiram treatment does not significantly alter DBH levels in CSF or plasma, although it increases sympathetic tone, plasma norepinephrine, and blood pressure. In the rhesus monkey, where the pool of plasma DBH is smaller, chronic disulfiram treatment induces a significant increase in plasma DBH which lasts for at least two months after drug treatment is stopped; there is no significant effect on CSF DBH. Chronic treatment of depressed patients with monoamine oxydase inhibitors (MAOI) has been shown previously to cause a decrease in CSF DBH. When MAOIs are given to cats, plasma DBH is significantly decreased after eight days of drug treatment, and CSF DBH is significantly decreased after twenty-two days of drug treatment. The MAOIs do not alter brain DBH levels. The results are consistent with a delayed decrease in central noradrenergic firing which occurs at the same time that therapeutic effects would be seen in humans. Treatment of cats with phenoxybenzamine causes an increase, and clonidine causes a decrease, in DBH in CSF. These results are consistent with the known α-antagonist and α-agonist properties of these drugs. The CSF DBH changes are not paralleled by plasma DBH changes. These data suggest that changes in CSF DBH may reflect changes in central noradrenergic activity.

Significance to Biomedical Research and Institute Program:

These studies show that intra-individual changes in CSF DBH activity may be useful as a marker of central noradrenergic activity and are generally independent of changes in sympathetic activity.

Proposed Course of Project:

DBH will be studied in CSF from psychiatric patients treated with other drugs which affect the central noradrenergic system. Laboratory animals will also be used to study the effects of new pharmacological treatments on DBH activity in CSF and plasma.

Publications:

Lerner, P., Major, L.F., Murphy, D.L., Lipper, S., Lake, C.R., and Lovenberg, W.: Dopamine-β-hydroxylase in human cerebrospinal fluid: Effects of monoamine oxidase inhibitors. Neuropharmacology 18: 423-426, 1979.

Major, L.F., Lerner, P., Ballenger, J.C., Brown, G.L., Goodwin, F.K., and Lovenberg, W.: Dopamine-β-hydroxylase in the cerebrospinal fluid: Relationship to disulfiram-induced psychosis. Biol. Psychiatry, 14: 337-344, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00122-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Urinary 3-Methoxy-4-hydroxy-phenylethylglycol in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP NIMH
Other:	Robert M. Post	Chief, Section on Psychobiology	BP NIMH
	James C. Ballenger	Clinical Associate	BP NIMH
	Alice Smith	Biological Lab Technician	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

3-Methoxy-4-hydroxy-phenylethylglycol (MHPG) is a major metabolite in norepinephrine which can be measured accurately in urine samples by a gas chromatographic assay. MHPG has been measured in urine from unmedicated depressed patients and normal volunteers. There was no significant difference between mean urinary MHPG values for the two groups.

PHS-6040
(Rev. 10-76)

Objectives:

This study was undertaken in order to establish baseline levels of urinary 3-methoxy-4-hydroxy-phenylethylglycol (MHPG) in unipolar and bipolar depressed patients and normal volunteers.

Methods:

Urinary MHPG was measured using a gas chromatograph equipped with an electron-capture detector. The analytical method was validated by comparison studies with standard samples tested by researchers in several different laboratories.

Major Findings:

The baseline MHPG values for the depressed patients were in close agreement with those reported by other groups. The urinary MHPG values for the normal volunteers were in a similar range. The mean urinary MHPG levels for the two groups were very similar, and there was a large variance for both groups.

Significance to Biomedical Research and Institute Program:

The amount of MHPG in urine may give an index of the turnover rate of norepinephrine, a neurotransmitter reportedly involved in affective illness.

Proposed Course of Project:

MHPG will be measured in urine from other depressed patients and normal volunteers and will be compared with other measures of noradrenergic activity, such as cerebrospinal fluid (CSF) MHPG, CSF norepinephrine, and plasma norepinephrine.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00123-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Blockade by Chronic Lithium Treatment of Presynaptic Dopamine Supersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dorothy W. Gallager	Senior Staff Fellow	BP NIMH
Other:	William E. Bunney, Jr.	Chief	BP NIMH
	Agu Pert	Psychologist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability of chronic lithium administration to modify tricyclic antidepressant-induced supersensitivity development in cells receiving 5-hydroxytryptamine input was investigated using microiontophoretic and single-cell recording techniques. Chronic tricyclic antidepressant administration for a period of 14 days resulted in a 5-fold increase in the sensitivity of hippocampal pyramidal cells to iontophoretically applied 5-hydroxytryptamine. This supersensitivity was not blocked by the concurrent administration of lithium.

PHS-6040
(Rev. 10-76)

Objectives:

To determine whether the blockade of supersensitivity development by lithium as previously demonstrated in the dopamine system could be generalized to other central nervous system amine systems.

Methods Employed:

Microiontophoresis and extracellular unit recording techniques.

Major Findings:

Supersensitivity development induced by chronic tricyclic antidepressant administration in cells receiving 5-hydroxytryptamine input is not blocked by concurrent administration of lithium.

Significance to Biomedical Research and Institute Program:

We have previously demonstrated that chronic lithium administration blocked presynaptic supersensitivity development in dopamine-containing neurons. This study demonstrates that blockade of supersensitivity by lithium may not be generalized to all central nervous system amine systems.

Proposed Course of Project:

The effect of chronic lithium treatment or subsensitivity of dopamine neurons and alterations in sensitivity in other central nervous system transmitter systems will be investigated.

Publications:

Gallager, D.W. and Bunney, W.E. Jr.: Effects of Chronic Lithium Administration on the Development of Supersensitivity in CNS Amine Systems: A Microiontophoretic Study. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. Oxford, Pergamon Press, 1979, in press.

Gallager, D.W. and Bunney, W.E. Jr.: Failure of chronic lithium therapy to block tricyclic antidepressant-induced postsynaptic 5-HT supersensitivity. Arch. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00124-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Lithium in the Treatment of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP	NIMH
Other:	Jack E. Rosenblatt	Staff Psychiatrist	SMRC	NIMH
	Candace B. Pert	Pharmacologist	BP	NIMH
	John F. Tallman	Biologist	BP	NIMH
	William E. Bunney, Jr.	Chief	BP	NIMH
	Louis Sokoloff	Chief	LCM	NIMH
	Robert M. Post	Chief, Section on Psychobiology	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology, NIMH; Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long-term treatment of rats with haloperidol produced an increased sensitivity to apomorphine in various brain regions as assessed by the [^{14}C]deoxyglucose method. Using this method only the zona compacta, lateral habenula and anterior cingulate cortex showed supersensitivity. Supersensitivity in all three regions was antagonized by concurrent treatment with lithium. Chronic treatment of rats with cocaine was found to induce both α- and β-adrenergic receptor supersensitivity. Concurrent treatment with lithium again antagonized this effect. Chronic lithium was also found to enhance cholinergic supersensitivity induced by chronic scopolamine. Lithium by itself appeared to enhance activity in the cholinergic system.

Changes in catecholamine receptor sensitivity have been postulated to underlie a number of pathologies in man. Tardive dyskinesia may follow or be intensified by the termination of chronic treatment with antipsychotic drugs. The development of tardive dyskinesia has been assumed to be the result of increased sensitivity of dopamine receptors. Oscillations in catecholamine receptor sensitivity have also been proposed to be a factor in the etiology of affective disorders, especially manic-depressive illness. We have previously found that chronic lithium treatment appeared to prevent the development of dopamine receptor supersensitivity. This series of studies was designed to further extend the analysis of this effect and to examine the effect of chronic lithium on other receptor systems.

[^{14}C]Deoxyglucose Analysis of Haloperidol and Lithium Interaction

The [^{14}C]deoxyglucose method (Sokoloff *et al*. J. Neurochem. 28: 897-916, 1977) was used to assess local glucose consumption in various brain regions following an injection of apomorphine in rats that had been pretreated chronically with either haloperidol, haloperidol and lithium, saline, or saline and lithium.

Effect of Lithium on Sympathomimetic-Induced Changes in α- and β-Adrenergic Receptor Sensitivity

Four groups of rats were treated chronically with 10 mg/kg cocaine HCl, cocaine and lithium, saline and lithium, or saline alone. Following three weeks of treatment the rats were sacrificed and their brains assayed for α-receptor binding using a modification of the method of U'Prichard *et al*. (Mol. Pharmacol. 13: 454-473, 1977) and β-receptor binding using a modification of the method reported by Tallman *et al*. (Proc. Nat. Acad. Sci. USA, 74: 873-877, 1977). Locomotor activity was also assessed in each group after 10 mg/kg of cocaine during the second treatment week. A similar study was conducted using 10 mg/kg amphetamine instead of cocaine.

Effect of Chronic Lithium on the Development of Cholinergic Supersensitivity

Four groups of rats were treated chronically with 50 mg/kg scopolamine, scopolamine and lithium, saline and lithium, or saline alone. Following three weeks of treatment the rats were all challenged with 10 mg/kg pilocarpine HCl, a cholinomimetic. The hypothermic response to pilocarpine was assessed 60 minutes and 90 minutes after injection.

Major Findings

Out of 44 brain structures only three showed supersensitivity to an apomorphine challenge following chronic treatment with haloperidol. Neurons in the zona compacta increased their metabolic activity following an injection of apomorphine, and this increase was further enhanced in animals that had been treated with haloperidol. Neurons in the lateral habenula and anterior cingulate cortex, on the other hand, decreased their metabolic activity. This decrease was further enhanced in rats that had been treated with haloperidol. Concurrent treatment of animals with lithium prevented the development of supersensitivity to apomorphine in all three brain regions. Lithium treatment by itself had no effect on the actions of apomorphine as assessed with the [^{14}C]-deoxyglucose method. These experiments again confirm our previous findings regarding the ability of lithium to prevent the development of dopamine receptor supersensitivity. Interestingly, although a number of structures changed their metabolic activity following apomorphine, supersensitivity was only found in three with this technique.

Rats that had been chronically treated with cocaine over two weeks showed an enhanced locomotor response to cocaine over rats that had not received chronic cocaine treatment. Animals that had received both cocaine and lithium did not show an enhanced response to cocaine, thus indicating that at least on the behavioral level, lithium was effective in antagonizing cocaine-induced supersensitivity.

At the receptor level chronic cocaine was found to induce both α- and β-receptor supersensitivity. Both of these effects were prevented by co-treatment with lithium. Lithium by itself also was again found to produce a slight subsensitivity in the β-adrenergic receptors.

Chronic administration of scopolamine increased the hypothermic effect of pilocarpine. Concurrent treatment with lithium appeared to potentiate this effect. Lithium by itself also seemed to enhance the effects of pilocarpine. One unusual finding from this study was the development of seizures in animals that had received a lithium diet and were subsequently challenged with 10 mg/kg of pilocarpine. Seizures could only be induced in naive rats with much higher doses of pilocarpine (750 mg/kg). These findings suggest that lithium increases the sensitivity of the muscarinic cholinergic system.

Significance to Biomedical Research and the Institute Program

Lithium is one of the most widely used and efficacious compounds in the treatment of mania and manic-depressive illness. Understanding its mechanism of action will aid in defining the factors underlying the expression of mania and manic-depressive illness.

Proposed Course

1. Further attempts will be made to analyze the effects of chronic lithium on other neurotransmitter systems. Special emphasis will be devoted to the enkephalinergic and GABA circuits.

2. A continued effort will be made to assess the effects of lithium on the development of sub- and supersensitivity in the GABAergic, dopaminergic and enkephalinergic systems.

3. An effort will be made to evaluate the effects of lithium on the several populations of dopamine receptors.

Publications

Bunney, W.E., Jr., Pert, A., Rosenblatt, J.E., and Pert, C.B.: Some Biological Considerations of the Mode of Action of Lithium in the Treatment of Affective Illness. In Kline, N.S., Gershon, S. and Schou, M. (Eds.): Proceedings of the International Lithium Conference: Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, in press.

Pert, A., Rosenblatt, J.E., Sivit, C., Pert, C.B., and Bunney, W.E. Jr.: Long-term treatment with lithium prevents the development of dopamine receptor supersensitivity. Science, 201: 171-173, 1978.

Pert, C.B., Pert, A., Rosenblatt, J.E., Tallman, J.F., and Bunney, W.E. Jr.: Catecholamine Receptor Stabilization: A Possible Mode of Lithium's Anti-Manic Action. In Usdin, E. (Eds.): Catecholamines: Basic and Clinical Frontiers. London, Pergamon Press, in press.

Rosenblatt, J.E., Pert, C.B., Tallman, J.F., Pert, A., and Bunney, W.E. Jr.: The effects of imipramine and lithium on α- and β-receptor binding in rat brain. Brain Res. 160: 186-919, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00143-04 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Physiological Function of Opiate Receptors and Their Endogenous Ligands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Donald L. Bowie	Chemist	BP NIMH
	Barbara Ewells	Chemist	IR NICHD
	Agu Pert	Psychologist	BP NIMH

COOPERATING UNITS (if any)

Intramural Research Program, NICHD

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 0.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors are also receptors for naturally occurring peptides called endorphins or enkephalins. Experiments in this laboratory are aimed at clarifying the identity and function of these peptides.

PHS-6040
(Rev. 10-76)

Objectives:

1. To obtain more information about the chemical identity of the opiate peptides with β-endorphin-like cross-reactivity which can be found in human and rat plasma and human and rat cerebrospinal fluid.

2. To gain a better understanding of opiate receptor function by defining two subcategories of opiate receptors.

3. To demonstrate physiological stimuli which produce large alterations in opiate ligand levels in plasma and cerebrospinal fluid.

Methods Employed:

1. Radioimmunoassay of β-endorphin-like immunoreactivity found in plasma carried out with a number of different antibodies which recognize different parts of the β-endorphin molecule.

2. Receptor binding of radiolabeled alkaloid and peptide opiates to rat membranes.

3. Radioreceptor assay of opiate peptides which retain physiological activity.

Major Findings:

1. Levels of endogenous opiate peptides and β-endorphin immunoreactivity are elevated in the pituitaries of obese rats (*fa*/*fa*) and obese mice (*ob*/*ob*).

2. Lesions of the arculate nucleus which reduce β-endorphin levels in brain by over 90%, also are accompanied by a 20-30% weight gain in lesioned animals.

3. Opiate receptors in the brain and periphery appear to be found in two forms which are closely analogous to the dopamine receptor subtypes. Type 1 opiate receptors are coupled to adenylate cyclase, are sensitive to sodium, and are found in analgesic areas of rat brain and in the guinea pig ileum. Type 2 opiate receptors are unaffected by GTP and are found in areas in the limbic system, the *vas deferens* and neuroblastoma-glioma cells. Type 1 receptors are much more sensitive to morphine than leu-enkephalin; in Type 2 receptors the sensitivity is reversed. Benzomorphans interact with both receptor subtypes.

4. Ejaculation in male hamsters produces about a 50-fold elevation of β-endorphin-like immunoactivity in their plasma.

Significance to Biomedical Research and Institute Program:

Opiates produce profound mood-altering, sleep-inducing and analgetic effects when administered to humans. These effects are due to an artificial mimicking of the natural effects of endogenous morphine-like factor(s) on their specific membrane opiate receptors. This new, previously undescribed neuronal and hormonal system, is a likely candidate for regulating mood, sleep, pain suppression and urinary output--four behavioral effects which exhibit pathological changes in mental illness. Detailed study of the biochemical functioning and brain distribution of "morphinergic neurons" may elucidate their role in normal and abnormal behavioral states.

Proposed Course:

We plan future experiments on the relationship between obesity and opiate peptides, including the assay of opiate ligands in human plasma, rat plasma and rat cerebrospinal fluid during various cycles of eating. We also plan further characterization of opiate ligands in clinical samples and their alterations during various psychological and physiological states.

Publications:

Allen, R.G., Herbert, E., Ninman, M., Shibuya, H., and Pert, C.B.: Coordinate control of corticotropin, β-lipotropin and β-endorphin release in mouse pituitary cell cultures. Proc. Nat. Acad. Sci. USA, 75: 4972-4976, 1978.

Chang, J.K., Shimizee, M., Fong, B.T.W., Peterson, W.J., Pert, C.B., and Pert, A.: ARG-B_h-endorphin: a synthetic β-lipotropin fragment with potent analgesic activity *in vivo*. FEBS Letters, in press.

LaMotte, C.C., Snowman, A., Pert, C.B., and Snyder, S.H.: Opiate receptor binding in Rhesus monkey brain: association with limbic structures. Brain Res. 155: 374-379, 1978.

Margules, D.L., Moisset, B., Lewis, M.J., and Pert, C.B.: β-Endorphin is associated with overeating in genetically obese mice (*ob*/*ob*) and rats (*fa*/*fa*). Science, 202: 988-991, 1978.

Pert, A., Pert, C.B., Davis, G., and Bunney, W.E. Jr.: Opiate Peptides and Brain Function. In van Praag, H.M. (Ed.): Handbook of Biological Psychiatry. New York, Marcel Dekker, 1979, Vol. II, in press.

Pert, C.B.: Opiate receptors and pain. Neurosci. Res. Prog. Bull. 16: 133-141, 1978.

Pert, C.B. and Bowie, D.L.: Behavioral Manipulation of Rats Causes Alterations in Opiate Receptor Occupancy. In Usdin, E., Bunney, W.E. Jr. and Kline, N.S. (Eds.): Endorphins in Mental Health Research. NewYork, MacMillan Press, 1979, pp. 93-104.

Pert, C.B. and Gulley, B.L.: The Mechanism of Opiate Agonist and Antagonist Action. In O'Malley, B.W. and Birnbaumer, L. (Eds.): Hormone Receptors: Peptide Hormones. New York, Academic Press, 1978, Vol. III, pp. 535-551.

Pert, C.B. and Taylor, D.P.: Demonstration and distribution of two opiate receptors in the brain and periphery. Proc. Nat. Acad. Sci. USA, in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00147-04 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral and Physiological Effects of Morphine-like Brain Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP NIMH
Other:	Dorothy W. Gallager	Senior Staff Fellow	BP NIMH
	Terry W. Moody	Guest Worker	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The actions of opiates and opioid peptides on opiate receptors located on neurons of the nigrostriatal dopamine system appear to increase dopaminergic activity by either enhancing release or acting in a synergistic manner with dopamine on post-synaptic sites. The cataleptic actions of opiates cannot, therefore, be due to blockade of dopamine transmission. Opiates act both in the periaqueductal gray matter and the nucleus gigantocellularis to produce analgesia. One effect of opiates at these brain levels is to activate descending inhibitory spinal pathways. These pathways appear to be coded, at least in part, by norepinephrine and serotonin. A number of peptides were found to be analgesic following microinjections into the periaqueductal gray matter. Among these were bombesin, neurotensin and bradykinin. Stress-induced analgesia was found to be partially antagonized by hypophysectomy and depletion of catecholamines.

PHS-6040
(Rev. 10-76)

Objectives:

The objectives of this project were to define the mechanism and sites of action of opiates and opioid peptides in brain and to evaluate possible physiological functions for these novel peptides.

Interactions of Opiates and Opioid Peptides with the Dopaminergic Systems in Brain:

Considering the striking similarities between the actions of opiates and classical neuroleptics on the release and metabolism of dopamine, it is not surprising that several investigators have proposed that the cataleptic actions of opiates may likewise be related to an inhibition of dopamine transmission in the striatum. These similarities led to the proposition that endorphins may actually be endogenous neuroleptics and that dysfunctions in the endorphin system may underlie the etiology of some mental disorders, especially those in which catatonia is a prominent symptom.

Recently, however, several important differences have emerged between these two classes of drugs which makes it unlikely that they share a similar mechanism of action. First, it appears certain that opiates do not inhibit dopamine transmission by interacting directly with postsynaptic dopamine receptors nor do they inhibit dopamine release by acting presynaptically. Second, the cataleptic actions of opiates appear to be determined predominantly through the periaqueductal gray matter while the cataleptic actions of neuroleptics appear to be mediated by the caudate nucleus.

To analyze the actions of opiates on the nigrostriatal system we employed the rotational model introduced by Ungerstedt (1971). This model is based on the principle that animals will rotate away from the striatum with the preponderance of dopamine activity. A dopaminergic imbalance is usually created by unilaterally lesioning the substantia nigra either electrolytically or by injections of 6-OHDA. Such lesions result in the degeneration of ascending dopamine neurons in the nigrostriatal pathway and the development of dopamine receptor supersensitivity in the ipsilateral striatum. Animals with unilateral substantia nigra lesions respond differentially to different classes of catecholaminergic drugs. Compounds which release dopamine from the terminals and prevent reuptake will make animals rotate ipsilateral to the lesion. Compounds which act postsynaptically as dopamine agonists will make animals rotate contralateral to the lesion due to their actions on supersensitive postsynaptic dopamine receptors.

In the first series of studies we evaluated the effects of acute and chronic morphine on rotational behavior in animals that had been lesioned unilaterally in the substantia nigra with 6-OHDA. In the second series of studies we assessed rotational behavior following unilateral injections of opiates and opioid peptides into various components of the nigrostriatal system. In the third series of studies we evaluated the effects of microinjections of morphine into the substantia nigra on dopamine metabolites in the striatum. Various lesion and pharmacological manipulations were made to evaluate the specificity of the opiate effects. In the electrophysiological analyses we examined the effects of morphine on the spontaneous activity of neurons in the zona compacta of the substantia nigra using both single-cell and iontophoretic recording techniques.

Analgesic Mechanisms of Action of Morphine in the Brainstem:

We have previously demonstrated that microinjections of morphine into the nucleus gigantocellularis and the periaqueductal gray matter of the rat brainstem will induce a potent analgesic reaction. This series of studies has been directed at ascertaining the precise mechanisms of action of morphine at this level of the brain. A great deal of emphasis has been placed on the activation of descending inhibitory spinal pathways by morphine. Some investigators have emphasized noradrenergic pathways while others have emphasized descending serotonergic pathways originating from the bulbar-raphe nuclei. The first series of studies, employing selective lesion techniques, was aimed at ascertaining the relative importance of the various brainstem noradrenergic and serotonergic nuclei in opiate analgesia. Rats were lesioned with either microinjections of 6-hydroxydopamine into the catecholamine nuclei (A-1 to A-6) or with 5,7-dihydroxytryptamine into brainstem raphe nuclei, and then assessed for morphine analgesia following systemic injections. In subsequent studies an attempt was made to ascertain whether opiates, acting directly in the nucleus gigantocellularis, induce at least part of their analgesic effects by attenuating brainstem serotonergic or noradrenergic pathways. Rats were implanted with microinjection cannulae in the nucleus gigantocellularis and then lesioned either in the catecholamine nucleus A-1 or in the raphe magnus as before. The effects of such lesions were evaluated on the analgesic actions of morphine following direct injections into the nucleus gigantocellularis. In order to ascertain whether the initial action of morphine in the nucleus gigantocellularis was inhibitory or excitatory, we employed both lesion and electrical-stimulation techniques. Morphine analgesia was assessed after lesions to nucleus gigantocellularis, and an attempt was made to ascertain whether analgesia could be induced with electrical stimulation of the nucleus gigantocellularis.

Pharmacological Activity of Non-Opioid Peptides:

Following the isolation and characterization of enkephalin in brain and the intensive neuropharmacological characterization of these peptides, there was an increase in interest regarding the functions of other neuropeptides. One peptide which appears to have quite potent actions in mammalian brain is bombesin, a tetradecapeptide isolated from frog skin. In this series we examined the regional distribution of bombesin receptors in brain utilizing brain punch and binding techniques. In subsequent studies we evaluated the effects of bombesin on nociception and locomotor activity. In further studies, neurotensin and bradykinin were compared to bombesin in the production of analgesia.

The Involvement of Endorphins in Stress-Induced Behavior:

Stress has been shown to increase the levels of both ACTH and β-endorphin in rat plasma. Several attempts have been made to relate these increases in circulating β-endorphin to various behavioral states. Since stress has recently been shown to induce an analgesic state, several attempts have been made to relate β-endorphin to stress-induced analgesia. We attempted to evaluate the involvement of endorphins in stress-induced analgesia using pharmacological as well as physiological techniques.

Major Findings:

Opiates appear to act both in the caudate nucleus and in the substantia nigra to increase dopaminergic activity. Systemic injections of morphine in rats that had received unilateral lesions of the substantia nigra produced ipsilateral rotational behavior indicating that systemic morphine is enhancing the release of dopamine in the intact nigrostriatal pathway. This effect was blocked by both naloxone and haloperidol. Direct injections of morphine into the substantia nigra induced contralateral rotational behavior indicating that at least part of the opiate effect on the nigrostriatal dopamine system was mediated directly through the substantia nigra. Knife cuts anterior to the injection blocked this effect as did injections of haloperidol directly into the ipsilateral caudate nucleus. The actions of morphine in the substantia nigra were also antagonized by pretreatment with amphetamine and naloxone. While these studies indicated that opiates were acting directly in the substantia nigra to increase dopamine activity, it was not clear whether this action was mediated through a direct effect on the zona compacta cells or through some other mechanism. Iontophoretic studies revealed that morphine does not directly excite the zona compacta neurons nor does it effect the inhibitory actions of dopamine on these cells. Studies are presently underway to ascertain the precise action of morphine in substantia nigra. Morphine was also found to affect the release of dopamine in the striatum. Amphetamine or morphine was found to produce contralateral rotations in rats that had been injected unilaterally in the caudate nucleus with morphine. Thus, it appears that enkephalinergic systems are intimately involved in regulating dopamine transmission at several levels in brain.

Lesions to catecholamine nucleus A-1 and raphe magnus were found to decrease dorsal horn norepinephrine 64% and serotonin 50% respectively. Even with these depletions there appeared to be only a relatively small effect on morphine analgesia following systemic administration. These findings seem to indicate that descending 5-hydroxytryptamine and norepinephrine pathways are not the only ones involved in opiate analgesia. The same lesions also failed to have a very significant effect on opiate analgesia following direct injections into the nucleus gigantocellularis. Stimulation of the nucleus gigantocellularis only produced post-stimulation analgesia with relatively high current levels. This apparent analgesia was not antagonized by naloxone. Lesions of the nucleus gigantocellularis appeared to slightly enhance morphine analgesia. These findings do not suggest that opiates induce analgesia in the nucleus gigantocellularis by an excitatory action.

The highest bombesin receptor content was found in the amygdaloid nuclei. Moderately high binding was also seen in the frontal pale of the brain containing the nucleus accumbens, olfactory nuclei and frontal cortex. The periaqueductal gray matter of the brain had a surprisingly high receptor content, especially in comparison to surrounding regions. The caudate nucleus, including part of the globus pallidus had moderate binding, while the thalamus, nucleus caudalis, nucleus gigantocellularis and cerebellum were low. Bombesin was found to induce a potent analgesic effect following injections into the periaqueductal gray matter. This was not antagonized by naloxone, indicating a dissociation from enkephalinergic mechanisms. Bradykinin and neurotensin also produced an analgesic effect following injections into the periaqueductal gray matter. The effects produced by bradykinin were relatively short lasting.

The main argument against the involvement of endorphins in stress-induced analgesia is based on the inability of naloxone to antagonize this effect. In addition, although stress does increase levels of β-endorphin in plasma, the levels are several magnitudes too low to account for the analgesic effect seen. Since we have been unable to see any effect of stress on cerebrospinal fluid levels of β-endorphin in rats, it does not seem likely that stress-induced analgesia is mediated by a central β-endorphin mechanism. On the other hand, hypophysectomy was found to partially reverse stress-induced analgesia, thereby suggesting pituitary involvement. We have recently found that pretreatment with amphetamine also partially antagonized stress-induced analgesia. Lesions to A-1 catecholamine nucleus or raphe magnus had no effect.

Significance to Biomedical Research and the Institute Program:

Since opiates are among the most potent psychomimetic and euphoragenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Furthermore, dysfunctions in the neuronal systems encoded by these peptides may underlie some of the etiology of mental disorders and neurological dysfunctions.

Proposed Course:

1. Attempts will be made to define the brain circuits and physiological conditions that regulate β-endorphin release from the pituitary.

2. Further attempts will be made to define environmental and physiological conditions that can modify enkephalin levels in brain.

3. Special emphasis will be directed at analyzing the effects of enkephalins on the mesolimbic dopamine system and the modulation of its activity by enkephalins and endorphins.

4. Efforts will be made to further define the descending brainstem pathways that are involved in morphine analgesia.

Publications:

Chang, J.K., Shimizee, M., Fong, B.T.W., Peterson, W.J., Pert, C.B., and Pert, A.: ARG-B_h-Endorphin: a synthetic β-lipotropin fragment with potent analgesic activity *in vivo*. *FEBS Letters*, in press.

Pert, A.: Central Sites Involved in Opiate Actions. In Fishman, J. (Ed.): *The Bases of Addiction*. Berlin, Dahlem Konferenzen, 1978, pp. 299-332.

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Pert, A. and DeWald, L.: Neurohormonal and neurohumoral modulation of stress-induced analgesia. *Neuroscience and Biobehavioral Reviews*, in press.

Pert, A., DeWald, L., and Gallager, D.W.: Effects of Opiates on Nigrostriatal Dopaminergic Activity: Electrophysiological and Behavioral Analyses. In Usdin, E. (Ed.): *Catecholamines: Basic and Clinical Frontiers*. London, Pergamon, in press.

Pert, A., DeWald, L.A., Liao, H., and Sivit, C.: Effects of Opiates and Opioid Peptides on Motor Behaviors: Sites and mechanisms of Action. In Usdin, E., Bunney, W.E. Jr., and Kline, N.S. (Eds.): *Endorphins in Mental Health Research*. New York, MacMillan Press, 1979, pp. 45-61.

Pert, A., Pert, C.B., Davis, G., and Bunney, W.E. Jr.: Opiate Peptides and Brain Function. In Usdin, E., Bunney, W.E. Jr., and Kline, N.S. (Eds.): *Endorphins in Mental Health Research*. New York, MacMillan Press, 1979, pp. 145-161.

Post, R.M., Davenport, A., Pert, A., and Squillace, K.: Lack of effect of an opiate agonist and antagonist on the development of amygdala kindling in the rat. *Research Communications in Psychopharmacology*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00155-02 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Brain Neuropeptide Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP	NIMH
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	Duncan P. Taylor	Guest Worker	BP	NIMH
	Agu Pert	Psychologist	BP	NIMH
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TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Receptors in brain membranes which mediate the pharmacological effects of the neuroactive peptides bombesin and vasointestinal peptide have been demonstrated by direct binding. The regional and subcellular distributions of these peptide receptors suggest that these receptors are physiologically important and that they may mediate important brain functions when they interact with endogenous components present in the central nervous system. Methods for detailed autoradiographic mapping of brain regional distribution of neurotransmitter receptors is being developed.

PHS-6040
(Rev. 10-76)

Objectives:

To understand the neuroanatomical distribution and functional significance of the various neuropeptide neuronal systems in brain.

Methods Employed:

1. Direct binding of radiolabeled peptides.
2. Radioimmunoassay and radioreceptor assay of neuropeptides.
3. Selective destruction of neuroanatomic pathways.
4. Microinjection of neuropeptides in various brain sites with concommitant assessment of behavioral alterations.

Major Findings:

1. Bombesin receptors in brain have similar steric requirements to the bombesin receptors found on pancreatic acinar cells.
2. Microinjections of bombesin in the periaqueductal gray matter produces antinociception in three rodent tests.
3. The endogenous ligand(s) for the bombesin receptor has been analyzed biochemically. The radioimmunoreactive material has a molecular weight similar to that of bombesin but a second brain component with a molecular weight of at least 3,000 appears to possess radioreceptor activity.
4. Neurons located in the ventral medial thalamus of the rat which project their axons into layers I and IV of the cortex probably secrete vasointestinal peptide (VIP) from their terminals.
5. A workable method for studying VIP receptors has been developed. As is the case with the bombesin receptor, the brain VIP receptor is similar to the pancreatic VIP receptor.
6. A method has been developed for the detailed autoradiographic mapping which is suitable for reversible ligands whether they are peptidergic or alkaloid in nature.

Significance to Biomedical Research and Institute Program:

It is simplistic, but certainly the discovery of what components make up the brain represents a most important advance in understanding how the brain works. Characterization of the distribution of new putative peptide neurotransmitter receptors can lead to the rational design of behavioral and neurophysiological experiments aimed at elucidating neurotransmitter function.

Proposed Course:

We plan to utilize selective lesioning techniques and immunohistofluorescence visualization of the neuropeptides themselves to try to understand the neuronal interconnections, particularly the thalamic-cortical projections.

Publications:

Jensen, R.T., Moody, T.W., Pert, C.B., Rivier, J.E., and Gardner, J.D.: Interaction of bombesin and litorin with specific membrane receptors on pancreatic acinar cells. Proc. Nat. Acad. Sci. USA, 75: 6139-6143, 1978.

Moody, T.W., Pert, C.B., Rivier, J., and Brown, M.R.: Bombesin: specific binding to rat brain membranes. Proc. Nat. Acad. Sci. USA, 75: 5372-5376, 1978.

Taylor, D.P. and Pert, C.B.: Vasoactive intestinal polypeptide: specific binding to rat brain membranes. Proc. Nat. Acad. Sci. USA, 76: 660-664, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00156-01 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Receptors in the Sympathetic Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
Other:	Nguyen Thoa	Pharmacologist	LCS NIMH

COOPERATING UNITS (if any)

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neurotransmitter receptors are being studied in the rat superior cervical ganglion. As neural transmission through the ganglion is modulated by catecholamines, variations in neurotransmitter receptors may accompany changes in transmission.

PHS-6040
(Rev. 10-76)

Objectives:

(1) To develop methods to measure the presence of neurotransmitter receptors in the superior cervical ganglion.

(2) To measure whether alterations in receptor number accompany alterations in nervous function in the ganglia.

Methods Employed:

The specific binding of tritiated α-adrenergic antagonist to membranes from the rat superior cervical ganglion is used to measure the number of α-adrenergic receptors. Ganglia are decentralized (pre-ganglionic fibers from the central nervous system severed) a week before the experiment, removed the day of the experiment and compared with intact ganglia freshly removed from the contralateral side of the rat.

Major Findings:

(1) Membranes from the rat superior cervical ganglion contain α-adrenergic receptors. [^{3}H]Dihydroergocryptine, an α-adrenergic antagonist, binds specifically, saturably and reversibly to the receptor. Specific binding is defined as that part of total binding displaced by a saturating concentration of phentolamine.

(2) Alpha-adrenergic agonists l-epinephrine and l-norephinephrine inhibit binding potently, whereas the β-adrenergic agonist l-isoproterenol is weak. Alpha-adrenergic antagonists phentolamine, dihydroergocryptine, and piperoxane are potent inhibitors of binding, and the β-antagonist dl-propranolol, a weak inhibitor. Binding of [^{3}H]dihydroergocryptine, then, is to an α-, not a β-, adrenergic receptor.

(3) Binding is stereoselective as d-norephinephrine is much less potent than l-norepinephrine in inhibiting binding.

(4) Binding is to α-adrenergic receptors as dopamine and serotonin are 1/100 as potent as l-epinephrine in inhibiting binding.

(5) Scatchard analysis suggests that specific binding is to one class of binding sites with a K_D of about 15 nM and the maximum binding at saturation of about 1.1 pmol/mg protein.

(6) Decentralization decreases the number of α-adrenergic receptors by 50% without a change in K_D, suggesting that the number, rather than the affinity of α-adrenergic receptors, is decreased by decentralization.

Significance to Biomedical Research and the Program of the Institute:

Membranes in the rat superior cervical ganglia contain α-adrenergic receptors. When the axons of cholinergic preganglionic fibers to the ganglion degenerate, 50% of the α-receptors are lost, suggesting that one-half of the α receptors are present on preganglionic endings located presynaptically with respect to the adrenergic neurons of ganglion. Pharmacological and physiological evidence has led to the suggestion that presynaptic α-receptors inhibit transmission through the ganglion by a negative feedback mechanism. These experiments add radioligand-binding evidence for the presence of α-receptors and suggest that at least half of the α-receptors are located on cholinergic preganglionic (presynaptic) nerve endings.

Proposed Course:

Alpha-receptors on ganglia will be measured during alterations in ganglion function. Also, other neurotransmitter receptors in the ganglia will be measured.

Publications:

Kafka, M.S. and Thoa, N.G.: Alpha-adrenergic receptors in the rat superior cervical ganglion. *Biochem. Pharmacol*. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00157-01 BP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies in Benzodiazepine Binding Sites in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Dorothy W. Gallager	Senior Staff Fellow	BP NIMH
Other:	Pierre Mallorga	Visiting Fellow	BP NIMH
	John W. Thomas	Chemist	BP NIMH
	M.Ayub K. Yousufi	Visiting Fellow	BP NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electrophysiological and receptor binding studies show that the benzodiazepines alter neuronal inhibition in the central nervous system through a GABA receptor/benzodiazepine/ionophore complex. Studies on the pharmacological implications for this complex interaction including GABAergic agents and anticonvulsants are described. In addition, studies on the membrane environment and the biochemical characterization of the isolated benzodiazepine binding site have been carried out.

PHS-6040
(Rev. 10-76)

Pharmacological Studies

Studies on the interactions of γ-aminobutyric acid (GABA) and benzodiazepines have been extended to include *in vivo* labeling of the benzodiazepine binding site in brain by injecting [^{3}H]diazepam intravenously. As previously reported for *in vitro* binding, the *in vivo* binding is enhanced by pretreatment of animals with the GABAergic agents amino-oxyacetic acid (AOAA) and muscimol.

Based on the anticonvulsant activity of the benzodiazepines and GABA-like agents and antagonism of the convulsant agents bicuculline and picrotoxin for benzodiazepine binding, the anti-epileptic agent diphenylhydantoin (DPH) (Dilantin) was investigated for its effects on the GABA-benzodiazepine system. Using extracellular recording and microiontophoretic techniques, DPH was found to increase the efficacy of benzodiazepines when tested on dorsal raphe neurons in brain. This increased biological effect could be correlated with enhanced specific binding of the benzodiazepine [^{3}H]diazepam. Increases in [^{3}H]diazepam binding are dose dependent following anticonvulsant doses of DPH, while not significantly altered by subconvulsant doses of DPH. In developing rats, effects on brain-specific [^{3}H]diazepam binding were found to parallel the time course for the development of anticonvulsant activity.

Benzodiazepine-Phospholipid Interactions

In an attempt to understand the events following the occupancy of the benzodiazepine receptor, the possibility that phospholipid metabolism might be altered by benzodiazepines was examined. Cultured astrocytoma cells having specific high-affinity binding sites for benzodiazepines as well as β-adrenergic receptors were used in these studies. Incubation of astrocytoma cells with both β-adrenergic agonists and benzodiazepines results in an additive increase in phospholipid methylation. The data indicate that β-adrenergic receptors and benzodiazepine receptors activate separate pools of phospholipid methylating enzymes and control separate domains of the membrane. Since methylation has been shown to increase membrane fluidity in the rat reticulocyte, it is suggested that an affect in membrane fluidity may be important in the functioning of the GABA/benzodiazepine/ionophore complex.

Benzodiazepine Binding Site Solubilization

To characterize the components of the GABA/benzodiazepine/ionophore complex in the membrane, recpeotr solubilization studies have been undertaken. The high-affinity binding site for benzodiazepines in mammalian brain has been solubilized using a non-ionic detergent. In a single step approximately 60% of the binding sites are solubilized, while maintaining a high affinity for [^{3}H]-diazepam (K_D of 11 nM as compared to 4 nM for the membrane-bound binding sites). The order of potency of benzodiazepines for the solubilized and membrane-bound binding is identical. With the soluble receptor, there is no GABA effect in [^{3}H]diazepam binding.

Significance to Biomedical Research and Institute Program:

These studies have been directed towards a more complete understanding of the pharmacology and biochemistry of the GABA receptor/benzodiazepine/ionophore complex. Based on electrophysiological and receptor binding studies, it is clear that the benzodiazepines alter neuronal inhibition in the central nervous system. The mechanism for this enhanced inhibition is obviously complex, but studies on membrane environment and on the biochemical characterization of the isolated binding site will lead to a more comprehensive understanding of the mode of action of benzodiazepines and, perhaps, brain mechanisms in general.

Proposed Course:

Continued studies on the function of the benzodiazepine binding site in neuronal inhibition are expected. We also hope to further characterize the isolated binding site and membrane environment of this receptor.

Publications:

Gallager, D.W.: Benzodiazepines: potentiation of a GABA inhibitory response in the dorsal raphe nucleus. Eur. J. Pharmacol. 49: 133-143, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00158-01 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Benzodiazepine Receptors and Their Endogenous Ligand

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Tomasso Costa	Guest Worker	ERR NICHD
	David Rodbard	Senior Investigator	ERR NICHD
	Laura Russell	Student Scientist	BP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Receptors in brain membranes which mediate the pharmacological effects of the benzodiazepines are apparently coupled to chloride ion channels, as revealed by the specific effect of anions on [^{3}H]diazepam binding. Analogies with opiate, glycine and GABA receptors suggest that the brain contains an endogenous component with the opposite pharmacological properties of valium.

PHS-6040
(Rev. 10-76)

Objectives:

To develop an understanding of the subcategories of benzodiazepine receptors based upon their ability to share the chloride ionophore with other neurotransmitter receptors.

Methods Employed:

Direct binding of tritiated benzodiazepine analogs.

Major Findings:

1. Benzodiazepine receptors are coupled to chloride ion channels which are shared by different neurotransmitter receptors in different brain regions.
2. The specific chloride anion channel blocker 4,4'diisothiocyano-2,2'stilbene disulfonic acid reverses the effects of chloride anions on benzodiazepine receptor binding.
3. It is feasible to develop conditions for detecting the binding of the putative brain component with properties opposite that of valium.

Significance to Biomedical Research and Institute Program:

The benzodiazepine receptor reveals many interesting new properties of neurotransmitter receptors. Its detailed understanding might lead to knowing how neurotransmitters regulate ionic flux when they interact with their receptors. The understanding of the brain's neuronal system which either mimics or antagonizes valium will certainly be relevant to the biochemical basis of human anxiety.

Proposed Course:

We intend to use the specific ion effects on diazepam binding, which we previously found, as a test of specificity for detecting an endogenous ligand for the valium receptor.

Publications:

Costa, T., Rodbard, D., and Pert, C.B.: Is the benzodiazepine receptor coupled to a chloride anion channel? Nature, 277: 315-317, 1979.

Costa, T., Rodbard, D., and Pert, C.B.: Interaction between the Benzodiazepine Receptor, the GABA Receptor and a Common Anion Channel. In DeLisi, C. and Blumenthal, R. (Eds.): Physical-Chemical Aspects of Cell Surface Events in Biological Regulation. Amsterdam, Elsevier/North Holland, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00326-06 CN

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical neuropharmacology and psychobiology of mania and depression.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
	Robert M. Cohen	Clinical Associate	CN NIMH
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	Leslie F. Major	Staff Psychiatrist	CN NIMH
	David Pickar	Clinical Associate	CN NIMH
	Larry J. Siever	Clinical Associate	CN NIMH
COLLAB:	Monte S. Buchsbaum	Section Chief	BP NIMH
	Edward F. Donnelly	Psychologist	SMR NIMH
	Frederick K. Goodwin	Chief	CP NIMH
	Daniel P. van Kammen	Section Chief	BP NIMH
	Herbert Weingartner	Acting Chief	LPP NIMH

COOPERATING UNITS (if any)

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Clinical Center Rehabilitation

LAB/BRANCH Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 8.4	PROFESSIONAL: 5.2	OTHER: 3.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In our recently completed study of monoamine oxidase (MAO)-inhibiting antidepressants, clorgyline (a selective inhibitor of MAO type A *in vitro*) was found to maintain its selectivity in man over the 4-week clinical trial period. The production of deaminated metabolites from the MAO-A substrates norepinephrine and serotonin was markedly reduced, while no changes in the MAO-B enzyme (which was directly measured in platelets) occurred. Pargyline, in contrast, totally inhibited the MAO-B enzyme, but also inhibited MAO-A activity. Clorgyline proved to be a more effective antidepressant agent and also produced fewer side effects--suggesting that MAO-A inhibition is most closely associated with the antidepressant effects of MAO inhibitors, and that there may be therapeutic advantages to the use of selective MAO-inhibitors. Other studies of patients with affective disorders investigated (a) several approaches to predicting individual responsiveness to antidepressant agents, (b) personality features and symptoms such as anxiety and obsessional thinking in depressed patient subgroups, and (c) behavioral assessment measures used in depressed and manic patients.

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with schizoaffective and severe obsessive symptomatology, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients's self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch.

Major Findings:

Evaluation of Clorgyline and Pargyline as Antidepressants and Inhibitors of Monoamine Oxidase (MAO) Types A and B in Man. This study, which is in its final stages, is an attempt to define whether any greater therapeutic benefit (either greater efficacy or fewer side effects) might be obtained from clorgyline compared to pargyline. These two MAO-inhibitors are structurally similar propargylamine compounds, but manifest differential inhibitory potencies on the two forms of MAO *in vitro*. There have been only a handful of studies examining whether these inhibitors yield selective effects *in vivo*, or whether any functionally significant selective effects are maintained *in vivo* with long-term drug administration.

In the last year, Dr. Campbell demonstrated that selective effects could be maintained with continued intraperitoneal administration of these drugs over an extended time period when low doses (0.5-1.0 mg/kg) were used in rats. After two or three weeks treatment, clorgyline (1.0 mg/kg/day) inhibited the deamination of serotonin (an MAO-A substrate) 100%, while the deamination of phenylethylamine, an MAO-B substrate was inhibited only 22%; pargyline (1.0 mg/kg/day) maintained somewhat less selectivity, but still yielded 90-93% inhibition of phenylethylamine deamination with 40-45% inhibition of serotonin deamination following two or three weeks' treatment.

In our studies in man, pargyline in doses averaging 87 mg/day (approximately 1.25 mg/kg/day) yielded 90-100% inhibition of platelet MAO activity beginning within hours of the first doses and continuing during the four-week treatment period. In contrast, clorgyline in doses averaging 28 mg/day (approximately 0.4 mg/kg/day) did not produce significant inhibition of the MAO-B in human platelets, even after four weeks' treatment. In contrast to their differential effects on platelet MAO-B activity, both drugs inhibited plasma amine oxidase activity (which is a different enzyme from the mitochondrial MAO-A/B enzyme forms) 42-67% after four weeks' treatment. Both drugs also produced similar reductions in systolic and diastolic blood pressures measured when the patients were standing.

This data was interpreted as indicating that clorgyline, in doses sufficient to produce hypotension and, as described below, definite behavioral effects, appeared to maintain selective effects in failing to inhibit the MAO-B in human platelets which has previously been shown to be sensitive *in vivo* to not only the selective MAO-B inhibitors pargyline and deprenyl, but *also* to non-selective MAO inhibitors including phenelzine and tranylcypromine. In subsequent studies, we have observed that lower pargyline doses (30 mg/day, equal to the clorgyline doses) and (-)-deprenyl, 10 mg/day, also produced complete inhibition of the platelet enzyme.

To further evaluate the biochemical consequences of the administration of clorgyline and pargyline to man, the cerebrospinal fluid (CSF) concentrations of a series of metabolites of dopamine, serotonin and norepinephrine were examined prior to and during the fourth week of administration of these drugs.

In these studies coordinated by Dr. Major, CSF concentrations of homovanillic acid (HVA), the major dopamine metabolite, were reduced following treatment with both drugs, but to a significantly greater extent with pargyline (65%) than with clorgyline (35%). Pargyline also tended to reduce the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) more than did clorgyline. The concentrations of the major serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) were moderately reduced by both clorgyline (45%) and pargyline (35%).

Cerebrospinal fluid levels of norepinephrine were not altered by either drug, while the concentrations of the major deaminated metabolite of norepinephrine, 3-methoxy, 4-hydroxy phenylglycol (MHPG) were markedly reduced during both the clorgyline and pargyline treatment periods. Small but statistically significant reductions in dopamine-β-hydroxylase (DBH) activity, but not in 3-methoxy, 4-hydroxymandelic acid (VMA) concentrations, were also observed in cerebrospinal fluid during clorgyline and pargyline treatment.

The greater reductions in HVA and DOPAC in CSF found with pargyline versus clorgyline treatment are consistent with *in vitro* data indicating that in man and in non-human primates dopamine is *primarily* metabolized by brain MAO-B (in contrast to the rat, in which dopamine is deaminated principally by MAO-A). The marked reductions in MHPG and 5-HIAA by clorgyline provide direct evidence that the clorgyline doses used were sufficient to inhibit MAO-A.

The similar magnitude of changes in MHPG and 5-HIAA produced by pargyline suggest that pargyline led to MAO-A as well as MAO-B inhibition -- although it is of note that in human brain and non-human primate brain studied in vitro both serotonin and norepinephrine are deaminated to a significant extent (30% or more) by MAO-B.

Although the dopamine metabolite changes provided the only evidence for a differential effect of the two inhibitors in the studies of cerebrospinal fluid, our studies of plasma norepinephrine levels (in conjunction with Dr. Lake) demonstrated larger and statistically-significant reductions in plasma norepinephrine levels during clorgyline treatment, in contrast to smaller and statistically non-significant changes observed with pargyline. Additional evidence for selective clorgyline vs. pargyline effects came from studies of urinary phenylethylamine excretion accomplished with Drs. Karoum and Wyatt. During pargyline administration, urinary phenylethylamine levels increased 100-fold (to 660 μg/24h) over baseline levels (6.7 μg/24h) in the nine patients studied; in contrast, negligible changes followed clorgyline treatment.

Conclusions Regarding Clorgyline and Pargyline as Selective MAO-A and MAO-B Inhibitors in Man. It would appear, then, on the basis of direct studies of MAO-B in platelets and of amines and their metabolites in cerebrospinal fluid, plasma and urine that some contrasting effects of clorgyline versus pargyline occurred during their chronic administration to man.

Clorgyline treatment was associated with an apparently selective inhibition of the MAO-A enzyme, with minimal inhibition of MAO-B -- as indicated principally by its marked effects in reducing CSF MHPG and 5-HIAA concentrations without altering platelet MAO activity or urinary phenylethylamine excretion.

In contrast, pargyline treatment clearly inhibited MAO-B activity, as indicated directly by the complete inhibition of platelet MAO activity and the marked changes in urinary phenylethylamine excretion. Pargyline also had a greater inhibiting effect on brain dopamine metabolism than did clorgyline, as indicated by the greater reductions in cerebrospinal fluid HVA and DOPAC concentrations which were observed during pargyline administration.

Pargyline, however, also produced reductions in cerebrospinal fluid 5-HIAA and MHPG, and although some portion of the changes might conceivably have resulted from the inhibition of MAO-B -- which can deaminate serotonin and norepinephrine to some extent in human brain -- it would seem most likely that MAO-A was also inhibited to a significant extent by pargyline. These findings are consistent with studies with human and rat brain showing that the MAO-B inhibitors manifest less relative selectivity than does the MAO-A inhibitor clorgyline in vitro. This difference may well account for their lesser selectivity seen both in studies in animals and our studies in man comparing MAO-B with MAO-A inhibitors.

The results from the behavioral assessment measures during the 4 week, double-blind, random assignment study of clorgyline compared to pargyline in 16 depressed patients yielded clear evidence that the two drugs had different clinical effects. In the initial comparative antidepressant efficacy study set up by Dr. Lipper, the patients receiving clorgyline all demonstrated reduced Hamilton depression scale ratings during the trial, with a mean difference that was statistically significant. Similarly, antidepressant and antianxiety effects and improved functional capacity were reported by the clorgyline-treated patients, with all six subscales of the NIMH self-rating scale yielding statistically significant differences.

In contrast, the patient group receiving pargyline remained essentially unchanged during the period of treatment. There was no indication of improvement on the Hamilton scale and little evidence for change on the NIMH self-rating subscales.

To evaluate more specifically whether the general behavioral effects of clorgyline differed from those of pargyline, the rating scale differences from the pretreatment placebo periods immediately preceding each drug trial versus the drug treatment period were compared in those patients who received both drugs in the crossover phase of the study. As before, clorgyline had apparently greater antidepressant and antianxiety effects than did pargyline, with significant differences between the two drugs observed on three of the subscales of the NIMH self-rating scale, on the total Brief Psychiatric Rating Scale (BPRS) and the BPRS anxiety-depression subscale, and on several POMS subscales and the IPAT anxiety subscales. Differences in the same direction, indicating greater improvement with clorgyline than pargyline, were also present on the Beck depression scale and on some of the MMPI scales.

Side effects of all types in the crossover study were more frequent and more severe in the pargyline-treated patients. Hypomania and postural hypotension occurred in equal frequency with both drugs. No hypertensive episodes occurred.

This data thus indicated that clorgyline, but not pargyline, exhibited significant antidepressant and antianxiety efficacy, according to the results from the self-rating and observer's scales in the patients who were included in the comparative efficacy trial. Similarly, the results of the crossover studies demonstrated that clorgyline was therapeutically superior to pargyline on every measure for which statistically significant differences were found between the two drugs studied in the same individuals.

On a probably simplistic level of interpretation, relatively selective changes in norepinephrine and/or serotonin metabolism could be postulated as the basis for the antidepressant effects in the clorgyline-treated patients. Such an interpretation would agree with evidence demonstrating that selective MAO-A inhibitors are more effective in reversing the sedative and locomotor effects of reserpine or tetrabenazine in one of the most widely used psychopharmacological animal models of depression. Thus, it may be that inhibition of MAO type A is more closely related to the antidepressant effects of MAO inhibition than is MAO type B inhibition.

Attempts to Predict Antidepressant Drug Responses by Biological and Psychological Measures. Acute activation and euphoriant responses to replicated single day trials with d-amphetamine proved to have moderately high correlations with subsequent antidepressant responses to imipramine and lithium carbonate during 3-4 weeks of treatment. These studies, initiated on 3-East when Dr. van Kammen was part of our group, indicate that common factors underly responsiveness to different drug groups in individual depressed patients. Preliminary data using MMPI sub-scales devised by Dr. Donnelly and Mr. Waldman suggest that predictive estimates of antidepressant responses to lithium and imipramine may also be obtained from pretreatment psychological tests. An extensive review of the question of the prediction of drug responses and the relationship of drug responses to diagnosis was completed in conjunction with Dr. Robin Murray of the Maudsley Psychiatric Institute during his sabbatical with our group a year ago.

Other Studies. Additional areas of investigation in patients with affective disorders include studies of personality features and of symptoms such as anxiety in subgroups of depressed patients, conducted in collaboration with Dr. Donnelly. Studies of memory and cognition in relation to mood state and drug effects are being conducted by Dr. Robert M. Cohen in collaboration with Dr. Weingartner. A series of projects to evaluate the assessment instruments and rating scales used in neuropharmacologic research in the affective disorders have been set up this year; a review of this area to be published in a book on psychiatric rating scales was recently completed.

Several additional measures of cognitive functioning in affective illness are being developed which include use of the Halsted-Reitan Battery, tachistoscopic laterality determinations, and computerized axial tomography. To augment study of receptor sensitivity changes, pupillometric measurements are being made with respect to mood changes and pharmacologic treatment effects. Allergic skin testing of individual patients will accompany this, as allergy-prone individuals have demonstrated increased adrenergic and cholinergic sensitivities.

Studies of monoamine-related enzymes and monoamine metabolites in platelets, plasma, cerebrospinal fluid and urine from depressed patients are described in other project reports from our Branch.

Publications:

Murphy, D.L., Campbell, I., and Costa, J.L.: The brain serotonergic system in the affective disorders. Prog. Neuro-Psychopharmacology 2: 1-31, 1978.

Donnelly, E.F., Murphy, D.L., and Goodwin, F.K.: Primary affective disorder: Anxiety in unipolar and bipolar depressed groups. J. Clin. Psychol. 34: 621-623, 1978.

van Kammen, D.P., and Murphy, D.L.: Prediction of imipramine antidepressant response by a one-day d-amphetamine trial. Am. J. Psychiatry 135: 1179-1184, 1978.

Carman, J.S., Gillin, J.C., Murphy, D.L., Weinberger, D.R., Kleinman, J.E., Bigelow, L.B., and Wyatt, R.J.: Effects of a selective inhibitor of type A monoamine oxidase--Lilly 51641--on behavior, sleep and circadian rhythms in depressed and schizophrenic patients. Commun. Psychopharmacol. 2: 513-523, 1978.

Greco, F.A., Oldham, R.K., Richardson, R.L., and Murphy, D.L.: Immunologic function in man receiving lithium carbonate. Biomedicine 29: 223-225, 1978.

Murray, R.M., and Murphy, D.L.: Can drug response contribute to psychiatric diagnosis and classification? Psychol. Med. 8: 667-681, 1978.

Donnelly, E.F., Murphy, D.L., Waldman, I.N., and Goodwin, F.K.: Prediction of antidepressant responses to imipramine. Neuropsychobiology 5: 94-101, 1979.

Murphy, D.L., Lipper, S., Slater, S., and Shiling, D.: Selectivity of clorgyline and pargyline as inhibitors of monoamine oxidases A and B in vivo in man. Psychopharmacology 62: 129-132, 1979.

Lipper, S., Murphy, D.L., Slater, S., and Buchsbaum, M.S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology 62: 123-128, 1979.

Post, R.M., and Murphy, D.L.: Research Planning: Studies of Cerebrospinal Fluid Metabolites and Blood Cell Enzymes. In van Praag, H.M. (Ed.): Handbook of Biological Psychiatry. New York, Marcel Dekker, 1979, Chap. 3, pp. 51-83.

Lerner, P., Major, L.F., Murphy, D.L., Lipper, S., Lake, C.R., and Lovenberg, W.: Dopamine-β-hydroxylase and norepinephrine in human cerebrospinal fluid: Effects of monoamine oxidase inhibitors. Neuropharmacology 18: 423-426, 1979.

Campbell, I.C., Shiling, D.J., Lipper, S., Slater, S., and Murphy, D.L.: A biochemical measure of monoamine oxidase type A and type B inhibitor effects in man. J. Psychiatr. Res. 10: 1-8, 1979.

Murphy, D.L., Lake, C.R., Slater, S., Lipper, S., Shiling, D., and de la Vega, E.: Plasma Catecholamines in Man During Treatment with Psychoactive Drugs. In Usdin, E., Kopin, I., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 918-920.

Major, L.F., Murphy, D.L., Lipper, S., and Gordon, E.: Effects of clorgyline and pargyline on deaminated metabolites of norepinephrine, dopamine and serotonin in human cerebrospinal fluid. J. Neurochem. 32: 229-231, 1979.

van Kammen, D.P., and Murphy, D.L.: Prediction of antidepressant response to lithium carbonate by a one-day administration of d-amphetamine in unipolar depressed women. Neuropsychobiology 5: 266-273, 1979.

Murphy, D.L., Pickar, D., and Alterman, I.S.: Methods for the Quantitative Assessment of Depressive and Manic Behavior. In Burdock, E.I., Sudilovsky, A., and Gershon, S. (Eds.): Quantitative Techniques for the Evaluation of the Behavior of Psychiatric Patients. New York, Marcel Dekker, 1979, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00327-07 CN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Marital Conflict of Manic-Depressive Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. F. Hoover	Social Worker	CN NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

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TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of conflict and dominance in marriage compares patients with primary affective disorders, their spouses, and individuals from the community.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of marital conflict and dominance in the lives of patients with primary affective disorders. Of special interest are levels of conflict, as well as characteristic patterns of decision-making and disagreement resolution.

Methods Employed:

The Conflict in Marriage Scale (CIMS), an agree-disagree card sort, was administered to 42 patients and their spouses, as well as to 30 comparable "well" marital pairs from the community.

Two forms of analysis were employed:

A. Responses indicating anger were assessed in relation to coping mechanisms; a "conflict ratio" for each individual and couple resulted. These ratios were then used for comparing levels of verbally-expressed marital conflict acknowledged by patients, their spouses and pairs from the community.

B. A discriminant function analysis was performed to isolate differences between patients, spouses, and community individuals on the basis of their CIMS responses, with special attention to those CIMS items relating to marital dominance and dependence.

Major Findings:

A. (1) Partnerships including a person with affective disorder reported significantly more conflict than community "well" pairs. (2) Patients reported significantly higher levels of marital conflict than their spouses did. (3) Conflict ratios between husband and wife in the marriages of patients showed virtually no correlation, although conflict ratios of community pairs were significantly correlated. (4) These results suggest that the spouses of patients with affective disorders may contribute to an evolving depression or mania by denying marital conflict of which the patient is aware.

B. (1) Ninety percent of patients and 47% of spouses were successfully divided in a two-way statistical discrimination on the basis of CIMS items. (2) One hundred percent of women patients could be sucessfully discriminated from women spouses. (3) All but one man could be correctly discriminated when men patients and men spouses were compared. (4) Spouses of patients were significantly more often reported to make final decisions on family matters than the patients were. (5) Based upon the report of wives from the three comparison groups, men patients were more likely than community men to leave family decisions to their spouses. (6) According to wives, women patients were also more likely to let their husbands make family decisions, than was true of community women.

Publications:

Hoover, C.F., and Fitzgerald, R.G.: Marital conflict of manic-depressive patients. Arch. Gen. Psychiatry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00328-04 CN

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Monoamine Oxidase Activity and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
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	Elliot S. Gershon	Section Chief	BP NIMH
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	Laurence M. Corash	Assistant Chief	CP NIH

COOPERATING UNITS (if any)

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Department of Psychiatry, Mayo Clinic (Dr. Elliott Richelson)

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIH, NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.2	PROFESSIONAL: 1.7	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is evaluating the associations between differences in monoamine oxidase activity and human or animal behavior. In continuing studies of individuals in the upper and lower 10% of MAO activity from a sample of 375 normals, rapid eye movement (REM) sleep and cortical evoked response differences have been shown to be associated with platelet MAO differences. Studies of factors capable of affecting platelet MAO have shown that smaller, lighter platelets have less MAO activity than larger, heavier platelets; however, most patients and normals with low MAO activity were not found to have a larger proportion of small, light platelets than controls. Marijuana smokers have lower platelet MAO activity than controls, but short-term THC administration or its addition to the enzyme in vitro did not reveal any significant inhibitory effect. These findings, in general, are consistent with our previous observations in patients and normals suggesting that reduced platelet MAO activity is associated with differences in personality features, psychobiological characteristics and a tendency towards increased vulnerability to psychopathology.

Project Description:

Objectives: This project investigates the apparent relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity and drug effects on MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project has also had as an objective the study of factors which affect platelet MAO activity and which may be relevant to the interpretations of differences in MAO activity found in psychiatric patient subgroups. The study of the mechanisms of action of drugs which inhibit MAO activity, and the consequences of MAO inhibition on amine neurotransmitter metabolism are additional objectives.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and other mammalian platelets, brain, heart, liver and kidney. Dr. Redmond has provided platelets and other tissues from vervet and rhesus monkeys as well as several other non-human primate species. Dr. Richelson has provided tissue culture preparations of C6 rat glial cells and mouse neuroblastoma cells. Dr. Wyatt has provided platelet samples from chronic schizophrenic patients, Dr. van Kammen samples from acute schizophrenic patients, and Drs. Coursey, Buchsbaum and Haier samples from normals. A large number of studies correlating MAO activity with clinical, biological (e.g., urinary MHPG, CSF metabolites, plasma norepinephrine and DBH) and pharmacological responses are being conducted on the 3-East Unit and collaboratively with other NIMH-IR investigators. Several different MAO assay procedures have been adapted or developed in this laboratory for the study of enzyme activity in platelets and other tissues. Techniques for the study of enzyme activity in different platelet populations have been worked out by Drs. Costa and Corash. Dr. Campbell has initiated investigations of the effects of chronic administration of selective inhibitors of the MAO A and B forms on neurotransmitter amines, amine metabolism and receptor function.

Major Findings:

Reduced Monoamine Oxidase Activity as a Vulnerability Factor for Psychopathology.

We have continued to explore the question of whether reduced monoamine oxidase activity might comprise a biological vulnerability factor for psychopathology. In a large majority of reports, chronic schizophrenic patients and patients with bipolar affective disorders and with alcoholism have been found to manifest reductions in platelet MAO activity. The reductions in the major psychiatric disorders are not explained by drugs or other known illness-related factors. Platelet MAO activity is a generally stable characteristic of the individual, and several twin and sibling studies have shown that genetic differences account for most of the inter-individual differences in activity. The well relatives of bipolar patients and, according to Dr. Major's study, the relatives of alcoholic patients also show equivalent reductions in MAO activity, suggesting that this difference represents a familial factor and is not the result of illness. Recent preliminary data from Dr. Gershon's patients suggest, however, that reduced MAO activity in family members of bipolar patients does not occur in parallel with illness in these families.

In explorations of the hypothesis of reduced platelet MAO activity as a vulnerability factor or as an index of such a factor, psychological test differences and the occurrence of psychosocial difficulties in students have been found to be associated with MAO activity differences. In continuing studies of the original group of 375 college students screened to find subgroups differing in platelet MAO activity, significant differences in amounts of REM sleep were found by Dr. Mendelson between the subgroups with the highest and lowest MAO activities. Another psychophysiologic measure, cortical evoked responses produced in response to different intensities of light, was also found to interact with platelet MAO activity differences in yielding better predictions than either variable alone of the likelihood of an individual to develop psychopathology. These biological indices support the extensive clinical and psychological test differences obtained in the low-high MAO subgroup comparisons in indicating that in a population of non-patients, platelet MAO activity differences are associated with psychobiological differences of relevance to psychopathology.

Further investigations of psychological test differences found in low vs. high MAO individuals by Dr. Donnelly led to the identification of MMPI subscales which appear capable of predicting whether individuals would fall into low or high MAO subgroups, and which were composed of items which fit rather closely to behaviors and personality features identified by other means in the low vs. high MAO individuals. Also using the MMPI data, Dr. Haier found significant associations between MAO activity and the MMPI paranoia subscale in affectively-ill patient groups, but not in the normal populations.

In studies of factors affecting platelet MAO activity, density gradient centrifugation with arabino-galactan was used to prepare four platelet fractions that differed in weight and volume. MAO activity in the lightest and smallest platelet subpopulation was approximately one-half that in the heaviest and largest subpopulation. Because platelet weight and size are thought to be related to platelet age, it now seems possible that some drug effects on platelet MAO activity might represent changes in platelet turnover. Factors other than platelet turnover rates must contribute to individual differences in platelet MAO activity, however, since individuals with markedly reduced platelet MAO activity from the low-high MAO population exhibited no shift in the proportion of lighter versus heavier platelets nor in the relative amount of MAO activity in each density gradient subfraction.

In another study to evaluate the possible association between reduced platelet MAO activity and drug use, platelet samples from individuals taking part in a study of marijuana smokers were examined. In 26 consecutively studied male marijuana smokers, MAO activity was found to be significantly lower than in a matched control group of non-smokers. However, no reduction in MAO activity was found when platelet samples were obtained before and immediately after the subjects smoked marijuana cigarettes containing 15 mg delta-9-tetrahydrocannabinol. Similarly, no _in vitro_ inhibition of platelet MAO activity was found until THC concentrations exceeding 100 times the peak values found in plasma after marijuana smoking were used, and even at 10,000 times peak plasma concentrations (10^{-3}M) of THC platelet MAO was only inhibited 35%. These results were interpreted as possibly indicating that chronic, but not acute marijuana usage could lead to platelet MAO activity reductions or, alternatively, that individuals with low MAO activity tend to use marijuana to a greater extent than other individuals--in keeping with our earlier reports of associations between low platelet MAO activity and higher scores on sensation seeking scales and certain forms of psychopathology commonly found in heavy drug users.

Laboratory Studies of MAO-A and MAO-B

Because our early studies indicated that platelet MAO consisted entirely of MAO type B, while brain contains both MAO-A and MAO-B, we have been attempting to understand the possible implications of a selective reduction in MAO-B vs. MAO-A for biogenic amine metabolism in the intact animal. To evaluate metabolic changes resulting from reductions in MAO activity, Dr. Campbell has been administering low doses of the selective MAO-A inhibitor, clorgyline, and the selective MAO-B inhibitor, pargyline, to rats. The inhibitory selectivity of these drugs towards serotonin deamination (MAO type A) and phenylethylamine deamination (MAO type B) can be maintained over a 21-day period by proper selection of low doses of these drugs (0.5-1.0 mg/kg/24h). The results are consistent with MAO type A catalyzing the deamination of serotonin and norepinephrine and with MAO type B having little effect on these monoamines. Dopamine appears to be deaminated in vivo principally by MAO type A in the rat (although in man and in monkeys, dopamine is primarily deaminated by MAO-B). Clorgyline administration during a 3-week period was accompanied by persistent elevations in brain norepinephrine concentrations; serotonin levels were also increased during the first 2 weeks, but returned towards control levels by the third week of treatment. Low doses of pargyline did not increase brain monoamine concentrations, but treatment with higher doses for 3 weeks led to elevations in brain norepinephrine and 5-hydroxy-tryptamine; at this time significant MAO-A inhibition had developed. The changes in monoamine metabolism seen at the end of the chronic clorgyline regimen are not due to alterations in tryptophan hydroxylase activity; at this time tyrosine hydroxylase activity was also unaffected. Ongoing studies are examining brain receptor changes and electrophysiologic accompaniments of chronic MAO inhibitor administration.

Publications:

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Murphy, D.L., Costa, J.L., Shafer, B., and Corash, L.: Monoamine oxidase activity in different density gradient fractions of human platelets. Psychopharmacology 59: 193-197, 1978.

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Wyatt, R.J., Potkin, S.G., Cannon, H.E., Buchsbaum, M.S., Murphy, D.L., Karoum, F., Gillin, J.C., and Stoff, D.M.: Phenylethylamine (PEA) and Chronic Schizophrenia. In Usdin, E., Kopin, I., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 1833-1835.

Gershon, E.S., Targum, S.D., Leckman, J.F., Guroff, J.J., and Murphy, D.L.: Platelet monoamine oxidase (MAO) activity and genetic vulnerability to bipolar (BP) affective illness. Psychopharmacol. Bull. 15: 27-30, 1979.

Campbell, I.C., Robinson, D.S., Lovenberg, W., and Murphy, D.L.: The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in the rat brain. J. Neurochem. 32: 49-55, 1979.

Wyatt, R.J., Potkin, S.G., and Murphy, D.L.: Platelet monoamine oxidase activity in schizophrenia. A review of the data. Am. J. Psychiatry 136(4A): 377-385, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00329-04 CN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Platelets as models for the study of neurotransmitter function.

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Clinical Neuropharmacology Branch

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The processes of amine uptake and amine storage, and the number and composition of platelet storage vesicles, are examined utilizing radiolabelled tracers, transmission electron microscopy, and electron microprobe X-ray analysis. Synchrotron radiation is used to study the fine structure of the calcium in various model systems. The studies have three principal objectives: (1) quantitation of amine uptake, and of the number and composition of storage vesicles in platelets following treatment with various psychoactive drugs and releasing agents; (2) evaluation of the mechanisms responsible for amine movement across membranes; (3) examination of the chemical and physical environment of storage vesicles.

Project Description:

Objectives: Previous work on this project has documented the utility of platelets as models for the study of neurotransmitter-related functions, and has examined the accumulation, release, and metabolism of biogenic amines by these cells. Work in this project period continues the evaluation of platelet aminergic functions, with special attention to experimental protocols and techniques which can provide insight into the chemical, physical, and molecular bases for many of these processes.

Studies Implemented: (1) Examination of changes in platelet vesicular (storage-granule) content and amine uptake which occur during normal platelet aging and when normal platelet function is altered by drugs or releasing stimuli; (2) preparation and evaluation of the properties of biochemically-isolated storage vesicles; (3) study of alterations in amine uptake and storage induced by various drugs (the ionophore X537A, the fructose derivative of serotonin, and releasing agents such as fluphenazine, fenfluramine, and quinacrine); (4) investigation of the role of energy metabolism and pH gradients in amine uptake and storage; (5) the use of Synchrotron radiation to study the fine structure of calcium, a constituent of platelet storage vesicles.

Methods Employed:

1. General Preparative Procedures. Platelets are isolated from normal volunteers and patients receiving certain drugs. Cells may be stored under varying conditions, or separated by density-gradient centrifugation into fractions of differing physiologic ages. Storage vesicles are isolated biochemically by sucrose-gradient centrifugation of lysed platelets.

2. Examination of Amine Fluxes in Intact Platelets. Platelet vesicular serotonin (5HT) stores are specifically labelled by incubation with C^{14}- or H^3-5HT, and the disposition of the vesicular material is followed during the de novo uptake of extracellular 5HT or of agents known to cause release of vesicular 5HT.

3. Morphological and Analytical Electron-Microscope Studies. Dense bodies in air-dried platelet whole mounts are counted utilizing transmission electron microscopy. Thin sections of conventionally-prepared platelets are photographed, and the dimensions of the plasma membrane and various intracellular structures are quantitated using stereological techniques. The calcium and phosphorus content of individual dense bodies in normal and drug-treated platelets, and following biochemical isolation, is measured by electron-probe X-ray microanalysis.

4. The Structure of the Calcium Absorption Edge Examined with Synchrotron Radiation. Calcium-containing solids and solutions, some of which serve as putative model systems for platelet dense bodies, are exposed to Synchrotron radiation. The absorbance spectrum of calcium X-rays is recorded and subjected to computer processing to extract information about the chemical and physical environment surrounding a typical calcium atom.

Major Findings:

1. Changes in Platelet Dense-Body Content Following Various Stimuli. "Exocytotic" releasing stimuli (viz. thrombin, the calcium ionophore A23187, ADP, drying and re-hydration) elicit characteristic responses from platelets depending on the presence or absence of plasma proteins or other wetting agents, platelet trauma during various types of storage, and the physiologic age of a platelet population. In vivo treatment with aspirin (to inhibit prostaglandin synthesis) appears not to alter normal release patterns.

2. Evaluation of Possible Changes in Dense-Body Composition Following Biochemical Isolation. Dense bodies maintain their membrane integrity and content of calcium and phosphorus following isolation in a medium containing high concentrations of agents which chelate calcium (viz. EDTA). Preparations of isolated dense bodies should thus be useful for studies of amine uptake and storage in a cytoplasm-free system, and may help to clarify the role of platelet cytoplasmic constituents in maintaining normal uptake and storage processes.

3. Effects of Amine Releasing Agents on Amine Uptake and Amine Content. A number of substances, including the psychoactive drugs fluphenazine and fenfluramine, have been shown to enter platelet storage vesicles and displace some portion of their 5HT content. As a model for this process, the intra-vesicular binding and exchange of quinacrine with vesicular 5HT has been examined in detail utilizing radiolabelled 5HT, quinacrine, and analogues of quinacrine (the latter measured by sensitive fluorescence assays). Quinacrine apparently occupies both some fraction of the intra-vesicular binding sites which are normally occupied by 5HT and a separate class of sites not available for 5HT binding. Nevertheless, 5HT can re-enter and bind inside vesicles containing large quantities of quinacrine. A second type of releasing agent is typified by X537A, which mediates the rapid release of intra-vesicular 5HT (but not tryptamine) and does not permit its re-accumulation in vesicles.

4. Interactions of the 5HT Uptake Site with 5HT Analogs. Although 5HT analogs bearing lipophilic N-terminal moieties can apparently enter platelet cytoplasm and vesicles, the addition of a hydrophilic fructose residue at the N-terminal position completely prevents uptake. Since N-terminal fructo-5HT binds effectively to the plasma-membrane uptake site for 5HT, however, uptake may not be mediated through a water-filled or hydrophilic pore.

5. Role of pH and Energy Availability in 5HT Accumulation. Platelets will take up extracellular 5HT present at an initial concentration of 10^{-10}M in the presence of cytoplasmic 5HT at a concentration of approximately 10^{-4}M. According to classical models for active transport, each 5HT molecule taken up would require the hydrolysis of 1 ATP molecule. Since 5HT uptake proceeds in the virtual absence of metabolic ATP, it seems likely that a classical model is inappropriate to characterize the platelet uptake system for 5HT. In addition, uptake apparently proceeds normally at an extracellular pH of 5.8, a phenomenon mitigating against a role for a proton-coupled transport mechanism.

6. Coordination Geometry and Chemical Environment of Calcium in Model Systems. As judged from the fine structure of the calcium X-ray absorption edge, aqueous solutions of calcium complexed to ATP, ADP and pyrophosphate have significant numbers of water molecules associated with each calcium atom. The calcium in solid calcium-ATP and calcium-pyrophosphate complexes, in contrast, appears to be more anhydrous. The spectra obtained from these preliminary studies will be used to evaluate complex model systems more closely resembling dense bodies, as well as preparations of intact platelets.

Significance to Biomedical Research and the Program of the Institute: The current studies extend the investigation of factors which regulate the uptake, storage, and release of 5HT by human platelets. Basic studies have increased our understanding of some of the physical and chemical bases underlying amine movement across the plasma and vesicular membranes, and for the sequestration of calcium, nucleotides, and 5HT inside platelet vesicles. More applied studies have investigated the effect of a number of drugs, including psychoactive substances, on platelet serotonergic function.

Publications:

Costa, J.L., Murphy, D.L., and Smith, M.A.: Comparison of the X537A-induced ionophoric removal of serotonin and tryptamine from intact human platelets. Res. Commun. Chem. Pathol. Pharmacol. 22: 27-36, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00330-01 CN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Use of Electron Energy-Loss Spectroscopy to Study Aminergic Systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electron energy-loss spectroscopy is used to document the ultrastructural location of fluorinated molecules inside platelets and synaptosomes. The data provides information about the intracellular sequestration of fluorinated analogs of biogenic amines. Developmental work on the role of radiation damage in limiting the information obtainable, quantitation and image processing of elemental maps, and the possibilities for chemical analysis is being carried out.

Project Description:

Objectives: Previous work from this laboratory has documented the utility of electron energy-loss spectroscopy (EELS) for the ultrastructural localization of lithium and fluorinated molecules in biological systems. Current work has used EELS in basic studies which explore and expand its potential in studies of platelets and synaptosomes.

Studies Implemented: (1) EELS has been used to delineate the intracellular distribution of relatively high concentrations of difluoroserotonin, 2-fluoro-2-deoxy-D-glucose, and 2-fluoroadenosine (in platelets) as well as 5-fluorodopamine (in rat caudate synaptosomes). (2) The intracellular distribution of difluoroserotonin added to platelets during a relatively short incubation period (30 seconds) has been compared to that seen after longer incubation periods (30-60 minutes). (3) The feasibility of utilizing EELS to obtain information about the chemical state of compounds sequestered in cells has been studied by employing a series of substituted fluorobenzenes as model systems. (4) The limitations to analysis posed by electron-induced radiation damage have been explored, and methods of reducing this damage have been examined. (5) Methods for the computerized processing of EELS data are being developed.

Methods Employed:

General Preparative Procedures. Air-dried platelet whole mounts are prepared following incubation of cells under appropriate conditions. Synaptosomes are incubated, rapidly frozen, freeze-substituted, and embedded in plastic. Thin sections of the embedded synaptosomes are prepared by conventional ultramicrotomy. Model systems containing the various compounds to be studied are prepared by rapid air-drying or by slow drying inside collodion sandwiches.

Analytical EELS Studies. Biological material and model compounds are examined in microscopes equipped to perform EELS studies, and fluorine distribution is mapped for relatively large specimen areas (by obtaining fluorine-specific images). Selected microareas (approximately 100 Å in diameter) are probed in the stationary-spot mode so that EELS spectra can be obtained. Quantitation of specific peaks in spectra is carried out by computer processing of the raw data.

Major Findings:

1. Ultrastructural Distribution of Fluorinated Tracers in Platelets and Synaptosomes. 2-Fluoro-2-deoxy-glucose, which is taken up and phosphorylated by intact platelets, appears to be distributed uniformly throughout the cell cytoplasm. 2-Fluoroadenosine, in contrast, is taken up, converted to 2-fluoro-ATP, and appears to be more highly concentrated in discrete subcellular structures (probably mitochondria) than in platelet cytoplasm. Intra-platelet difluoroserotonin is highly concentrated in platelet dense bodies, even after a relatively short (30-second) exposure

period. Furthermore, each vesicle in a given cell contains fluorinated material regardless of the incubation time. 5-Fluorodopamine appears to be highly concentrated in synaptosomal vesicles in a similar fashion.

2. Model Systems for Evaluation of the Potential Utility of EELS for Chemical Analysis. Electron-induced radiation damage can lead to rupture of the chemical bonds in fluorinated tracer molecules and the subsequent loss of fluorine from the specimen. This phenomenon, which presents a potential limitation to the use of EELS for chemical analysis or with very high sensitivity, appears to be controlled if certain techniques for specimen preparation are employed.

3. Methods for Computer Processing of EELS Data. Developmental work done during the period of this report has indicated what capabilities will significantly enhance the utility of the EELS technique. The EELS facility in BEIB will have the capacity to record specific elemental images directly on magnetic tape. Coupled with appropriate processing of these images, it will be possible to extract spectral (quantitative) information directly from the images, thus greatly reducing the time needed to obtain data.

Significance to Biomedical Research and the Program of the Institute: The current work documents the utility of EELS for understanding the ultrastructural correlates of amine storage, as well as a variety of other parameters relevant to cellular function, in platelets and synaptosomes. With the greatly increased availability of instrument time provided by the new BEIB electron microscope facility, more specific studies can now be undertaken. In addition, further exploratory work on the use of EELS for ultramicro chemical analysis can continue by building on techniques developed during the current report period. It can be anticipated that extension of the EELS technique to many areas of biology will provide new insight into the dynamics of cellular function.

Publications:

Silbergeld, E.K., Adler, H.S., and Costa, J.L.: Subcellular localization of lead in synaptosomes. Res. Commun. Chem. Pathol. Pharmacol. 17: 715-725, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00331-01 CN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Use of Nuclear-Magnetic Resonance to Study Aminergic Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intact platelets and platelet extracts have been studied by biochemical analysis and by the nuclear-magnetic-resonance examination of fluorinated psychoactive drugs and amines, and phosphorylated compounds of low molecular weight. Lithium contained in model systems, platelets, and red blood cells has also been studied by nuclear-magnetic-resonance. Some chemical and physical concomitants of drug, amine, and nucleotide storage have been determined, and correlated with differences in the composition of storage vesicles. The lithium resonance in model systems and cells appears to reflect the water content and relative motional restriction of the immediate environment.

Major Findings:

1. Comparison of Amine Uptake and Storage in Pig Versus Human Platelets. Pig-platelet vesicles contain serotonin plus ATP and magnesium, as opposed to the serotonin plus ATP, ADP, pyrophosphate, and calcium found in vesicles of human platelets. Although pig platelets contain on the average 4-5 times more vesicles than do human platelets, each human vesicle can store approximately the same amount of serotonin as a pig vesicle. In pig vesicles, both ATP and serotonin (as judged from the behavior of difluoroserotonin) appear to exist in a gel-like state, perhaps tightly complexed together, whose molecular tumbling rates increase linearly (by about 2 orders of magnitude) as the temperature is raised from 4°C to 37°C. In human vesicles, the adenine nucleotides and pyrophosphate appear equally restricted in motion at both 4°C and 37°C (at least 4 orders of magnitude more than if they were in free solution). It has not yet been possible to see if difluoroserotonin is similarly restricted, since this compound is lost rapidly from the cells at temperatures above 22°C. Human-platelet vesicles can be filled with serotonin to their maximal capacity, or emptied of essentially all their serotonin, without apparent change in the vesicular phosphorus residues. Vesicles in pig platelets, in contrast, appear to lose most of their ATP when the serotonin is removed. It is of interest that unlike human platelets, pig platelets appear not to accumulate cytoplasmic (extra-vesicular) amines, and in addition display relatively much slower rates of amine uptake across the plasma membrane.

2. NMR of the Lithium Nucleus in Model Systems, Red Blood Cells, and Platelets. As determined from studies of both model systems and cells containing lithium, the lithium-7 nucleus is both readily detectable at biological concentrations (possible sensitivity better than 10^{-4}M) and a moderately good indicator of the chemical and physical parameters existing in its immediate environment. When lithium enters red blood cells following incubation at 37°C in a lithium-containing buffer, the lithium appears to exist in an uncomplexed form in an environment which is largely aqueous. Changes in the amount of intracellular water, however, produce alterations in the NMR-measurable parameters of the lithium nucleus. Preliminary evaluation shows that lithium accumulates in significant quantities inside platelets, and should be amenable to NMR study in this system.

Significance to Biomedical Research and the Program of the Institute: These studies characterize certain features of the amine storage complex in intact pig and human platelets. Dramatic differences between the vesicles of the two species indicate that a variety of storage mechanisms exist, and underscore (1) the uniqueness of the human platelet and (2) the inadvisability of using platelets from other animals as models for the human platelets. Future NMR studies should permit (1) a more detailed understanding of amine storage in human platelets, (2) documentation of changes in the amine storage complex after releasing agents, psychoactive drugs (i.e., fluphenazine, fenfluramine), and (3) more detailed evaluation of the intracellular environment of psychoactive drugs and the lithium ion.

Project Description:

Objectives: The current studies have employed nuclear-magnetic-resonance (NMR) techniques to provide chemical and physical information about amine uptake and storage processes in intact human platelets. In many cases, morphological (electron-microscopic) and more conventional biochemical experiments (with radiolabelled tracers) have been done in parallel with the NMR studies. Data obtained from the morphological and biochemical studies can be correlated with the NMR data, and is used to relate this data to our understanding of platelet function based on previous biochemical and morphological work (see Projects Z01 MH 00329-04 and Z01 MH 00330-01).

Studies Implemented: (1) Examination of the activation energies, molecular tumbling rates, and chemical-shift parameters of storage-vesicle contents (difluoroserotonin and adenine nucleotides) in intact human and pig platelets. (2) Evaluation of changes in NMR-measurable parameters following treatment of human and pig platelets by procedures known to alter the composition of the storage vesicles. (3) Biochemical and morphological studies to define better the striking differences seen by NMR between pig and human platelets. (4) NMR examination of the lithium-7 nucleus in aqueous solutions, after the incorporation of lithium into red blood cells and platelets, and in red blood cells and platelets from psychiatric patients on chronic lithium therapy. (5) Determination of the suitability of various fluorinated amines and psychoactive substances for study by NMR, and the design of suitable protocols and questions to be addressed in such a study.

Methods Employed:

1. General Preparative Procedures. Platelets are isolated from normal volunteers and psychiatric patients on long-term therapy with lithium or fluphenazine by plateletphoresis or serial differential centrifugation; red blood cells are isolated by differential centrifugation. Pig platelets are obtained by total exsanguination and differential centrifugation. Cells are incubated according to various protocols and prepared either for biochemical and morphological examination (by incubation with radiolabelled tracers or preparation of air-dried whole mounts) or for NMR (by resuspending cells at a very high cell density).

2. Biochemical and Morphological Studies. The distribution of labelled and endogenous amine between cytoplasm and vesicles is made by using thrombin or the calcium ionophore A23187 to release vesicles preferentially. Dense bodies are counted by electron microscopy, and their elemental composition is evaluated by electron microprobe X-ray analysis.

3. NMR Studies. Platelets and red blood cells are examined in NMR spectrometers of varying field strengths, employing appropriate pulse sequences, in order to assess properly the molecular tumbling rates. In addition, cells are examined by NMR after various procedures known to alter cellular cytoplasmic viscosity, energy metabolism, vesicular composition, or aminergic function. In some cases, changes in NMR signals can be observed directly as a function of time during changes in the observed sample.

Publications:

Costa, J.L., Dobson, C.M., Kirk, K.L., Poulsen, F.M., Valeri, C.R., and Vecchione, J.J.: Studies of human platelets by ^{19}F and ^{31}P NMR. FEBS Letters 99: 141-146, 1979.

Costa, J.L., Dobson, C.M., Kirk, K.L., Poulsen, F.M., Valeri, C.R., and Vecchione, J.J.: Nuclear magnetic resonance studies of blood platelets. Proceedings of Royal Society - Series B (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00332-01 CN

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Animal models for the study of drug effects on CSF amines and their metabolites.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leslie F. Major	Staff Psychiatrist	CN NIMH
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	Iain C. Campbell	Visiting Scientist	CN NIMH
COLLAB:	Pauline Lerner	Staff Scientist	BP NIMH
	Michael H. Ebert	Section Chief	LCS NIMH
	Robert M. Post	Section Chief	BP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

SUMMARY OF WORK (200 words or less - underline keywords)

The development of methods for the periodic or continuous sampling of cerebrospinal fluid (CSF) in cats and monkeys has allowed us to investigate the effect of various noradrenergic drugs on norepinephrine (NE) concentrations and dopamine β-hydroxylase (DBH) activity in the CSF in animals.

PHS-6040
(12-75)

Project Description:

Objectives: Rhesus monkeys and cats are utilized to study the effects of various drugs on cerebrospinal fluid levels of neurotransmitters and their metabolites to better understand the results obtained from cerebrospinal fluid studies in humans.

Methods Employed:

1. Behavioral assessment: Detailed rating scales of activity, arousal, posturing, appetite, grooming, and aggressiveness were employed both prior to and during treatment with alcohol, tricyclics, MAO inhibitors and dopaminergic and noradrenergic agonists and antagonists.

2. Biological assessment: Plasma, platelets, and cerebrospinal fluid are collected for the measurement of levels of biogenic amines and their metabolites, enzyme activities, other chemical variables, and drug levels. Cerebrospinal fluid (CSF) is collected by means of cisternal taps and in some cases by means of indwelling ventricular or lumbar cannulas for continuous flow into a fraction collector.

Major Findings:

1. Evaluation of pargyline and clorgyline as inhibitors of monoamine oxidase A and B in cats. This study was an extension of similar studies done in both humans and in rodents. It was undertaken in an attempt to bridge the gap between human studies where only bodily fluids (CSF and plasma) are studied, and rodent studies in which only plasma and brain tissue are studied. In the cat study, plasma and CSF were examined both acutely and chronically and brain tissue analyzed at the termination of the drug trial. We were able to follow the aminergic changes in CSF over the course of the 3-week study and then compare these changes to those found in the brain upon termination of the study. The preliminary findings indicate that CSF DBH activity, which is known from our studies in patients to be reduced after 4 weeks' treatment with MAO-inhibiting antidepressants, does not change significantly until 18-21 days of treatment with the MAO inhibitors, approximately the time at which clinical response to these drugs is usually seen. These findings suggest that the clinical response to MAOI's is related not to simple inhibition of MAO, but to some secondary effect of inhibition on the aminergic systems, such as a receptor change.

2. Effects of alpha and beta noradrenergic agonists and antagonists on CSF NE-DBH in cats. Propranolol was shown to increase both DBH and NE in CSF while clonidine decreased both NE and DBH. These findings are consistent with the hypothesis that central alpha adrenergic activity controls peripheral blood pressure via its inhibitory control of sympathetic outflow.

3. Effect of imipramine on noradrenergic activity in the rhesus monkey as measured by levels of NE and DBH in CSF. Imipramine, a noradrenergic

uptake blocker, caused an increase in NE after 24 hours with a return to baseline by day 15 of treatment. CSF DBH activity showed similar changes in the opposite direction, indicating that CSF DBH levels may be a good indicator of the firing rate of central noradrenergic neurons, while NE activity in CSF reflects the combined effect of uptake blockade and firing rate changes. These results confirm our previous suggestions concerning the interpretation of changes in NE and DBH in the CSF of humans during antidepressant drug administration.

4. Effects of acute and chronic alcohol administration on aminergic activity in rhesus monkeys as measured by changes in CSF. This study has been going on for the last 12 months and has involved our developing an animal model for alcohol addiction in the rhesus monkey, and a technique for continuous CSF collection from either the lateral ventrical or the lumbar space. Continuous CSF samples have been collected from 6 monkeys after both acute and chronic alcohol administration and during withdrawal from alcohol. These samples are being analyzed for DBH, NE, prolactin, cortisol, vasopressin, cyclic AMP, GABA, serotonin, and melatonin, and should help in understanding the effects of both acute and chronic alcohol administration and the withdrawal from alcohol in several different systems in the CNS.

Publications:

Lerner, P., Dendel, P. S., and Major, L. F.: Dopamine-β-hydroxylase in cerebrospinal fluid and plasma: effects of α-adrenergic agents. Brain Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00333-01 CN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical neuropharmacology and psychobiology of alcoholism.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leslie F. Major	Staff Physician	CN NIMH
OTHER:	Dennis L. Murphy	Chief	CN NIMH
COLLAB:	James Hawley	Neurologist, Washington VA Hospital	
	Elliot Shullman	Neurologist, Washington VA Hospital	
	Gerald L. Brown	Staff Medical Officer	BP NIMH
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	C. Raymond Lake	Staff Psychiatrist	LCS NIMH
	Michael Ziegler	Univ. of Texas, Galveston	
	Edna K. Gordon	Chemist	LCS NIMH
	Pauline Lerner	Staff Scientist	BP NIMH
	Walter Lovenberg	Section Chief	HE NHLBI

COOPERATING UNITS (if any)

Neurology Service, VA Hospital
Psychiatry Service, NNMC

LAB/BRANCH
Clinical Neuropharmacolgy Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of clinical symptomatology in young healthy male alcoholics demonstrated a lack of the association between depression and alcoholism which has previously been reported. The alcoholic group was also found to have no more aggressive behavior than age-matched normal controls while having a higher aggressive attitude. Biochemical studies on platelet monoamine oxidase (MAO) found lower MAO activity in alcoholics. Disulfiram which is used as a regular part of alcoholism treatment programs was found to cause an increase in both serum cholesterol and plasma norepinephrine (NE) with an associated increase in blood pressures, raising the possibility that long-term disulfiram use might increase the risk of hypertension and/or cardiovascular disease. Disulfiram also precipitated psychosis in 8 individuals who were noted to have lower platelet and plasma MAO with higher red cells catechol-O-methyl transferase (COMT) and lower cerebrospinal (CSF) dopamine-β-hydroxylase (DBH) activity prior to taking disulfiram. Autopsy brain samples from alcoholics have higher GABA receptor activity and lower β-adrenergic receptor activity than control brains.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project investigated the relationship between alcoholism, depression and aggression, and the effects of disulfiram. It further investigated the role of enzymes of monoamine metabolism in alcohol withdrawal as well as providing markers for individuals with higher vulnerability to alcoholism. The effects of chronic alcoholism, alcohol withdrawal and treatment with disulfiram are also currently being investigated using platelets, plasma, red cells and CSF in living subjects and brain, liver, kidney, lung, muscle and spleen samples from autopsy specimens.

Methods Employed:

Platelet, plasma, and CSF samples were obtained from alcoholics at both the NNMC and VA hospitals and from control groups at each hospital. Behavioral ratings using the BPRS, Hamilton and Zung Depression and Anxiety Rating Scales, SCL 90, Buss-Durkee Aggression Scale, and MMPI were collected. Demographic information using the ECDEU scales and the RDC were also collected. Multiple autopsy specimens on alcoholics and controls were collected at the VA Hospital along with clinical and neuropathological findings.

Major Findings:

1. Platelet MAO activity in alcoholics. This study involved serial platelet MAO determinations in over 100 male alcoholics admitted to the Alcoholic Rehabilitation Unit at the National Naval Medical Center, Bethesda, MD. Compared to age-matched military controls, the alcoholics were found to have low platelet MAO activity. To differentiate a primary (genetic) from a secondary (caused by alcohol consumption) cause for low MAO, we further divided the alcoholic group based on family history of alcoholism. We found that those alcoholics with a first degree alcoholic relative had lower platelet MAO than the remainder of the group. This was true for the normal controls also, indicating that low platelet MAO may be a vulnerability factor for alcoholism. To more clearly delineate the effects of alcohol abstinence on platelet MAO, we looked at platelet MAO every 3 days in a group of 12 alcoholics. We found that platelet MAO increased by 60% over 5-7 days of abstinence, returning to baseline levels by 14 days. These findings emphasized the importance of controlling for length of abstinence in studies of platelet MAO activity or other platelet function studies.

2. The behavioral and biochemical effects of disulfiram (Antabuse) in humans. Disulfiram has been used in the treatment of alcoholism for over 30 years. Because of the lack of well controlled studies on either the behavioral or biochemical effects of disulfiram in man, we undertook a single blind placebo controlled study of disulfiram. We did not find that disulfiram had any anti-anxiety effects or that it exacerbated depression as measured by the Hamilton and Zung depression and anxiety scales. We did note that certain individuals receiving the high dose (500 mg/day) developed psychotic reactions to the drug. These individuals were noted in retrospective analyses to have had lower platelet and plasma MAO, higher red cell COMT and lower CSF DBH prior

to starting disulfiram than the group receiving the same dose of disulfiram without a psychotic reaction. These findings suggest that the enzymes of monoamine metabolism are important determinants of vulnerability to drug induced psychoses. Disulfiram was also noted to cause an increase in serum cholesterol and serum norepinephrine and blood pressure suggesting that long-term disulfiram use may have untoward effects not previously recognized. Several individuals were also noted to have mild subclinical hepatotoxic reactions to disulfiram that would usually go undetected because of their alcohol history.

3. The relationship of depression and aggression to alcoholism. Previous reports of a relationship between alcoholism and unipolar or bipolar depression led to this study carried out at the National Naval Medical Center. Because all of our subjects were young, healthy, active duty males, we did not have the sampling bias of many other studies which selected from a group of alcoholics admitted because of medical or psychiatric sequelae of alcoholism. We did not find a significantly higher incidence of depression in our group as compared to normal controls suggesting that the co-existence of depression and alcoholism in other studies may be a selection bias or a result of more ethanol consumption over a longer period of time. There was, however, a higher level of aggressive attitude in the alcoholics as measured by the Buss-Durkee aggression scale, without a concomitant increase in aggressive behavior as seen in anti-social personality disordered patients. This finding is consistent with the hypothesis that high levels of resentment and irritability without any behavioral outlet may lead to an increased incidence of alcohol abuse.

4. The noradrenergic system in severe alcohol withdrawal. CSF NE was assayed in 100 male alcoholics at the Washington VA Hospital. These individuals were in various stages of alcohol withdrawal when the CSF was obtained. CSF NE was found to be higher during withdrawal than in samples obtained weeks later. We are presently looking at c-AMP and MHPG to further assess the noradrenergic system.

5. Receptor activity and enzyme activity in brain, liver, lung, muscle, and spleen from alcoholics and non-alcoholics controls. Preliminary studies of brain tissue suggests that alcoholics have higher GABA receptor activity and lower β-adrenergic receptor activity as measured by the displacement of radioactive ligands. We are presently looking at MAO A and B activities in several brain regions in alcoholics and controls.

Publications:

Lake, C.R., Major, L.F., Ziegler, M.G., and Kopin, I.J.: Increased sympathetic nervous system activity in alcoholic patients treated with disulfiram. Am. J. Psychiatry 134: 1411-1414, 1977.

Major, L.F., and Murphy, D.L.: Platelet and plasma amine oxidase activity in alcoholic individuals. Br. J. Psychiatry 132: 548-554, 1978.

Major, L.F., and Goyer, P.F.: Effects of disulfiram and pyridoxine on serum cholesterol. Ann. Intern. Med. 88: 53-56, 1978.

Lerner, P., Goodwin, F.K., van Kammen, D.P., Post, R.M., Major, L.F., Ballenger, J.C., and Lovenberg, W.: Dopamine-β-hydroxylase in the cerebrospinal fluid of psychiatric patients. Biol. Psychiatry 13: 685-694, 1978.

Major, L.F., Murphy, D.L., Gershon, E.S., and Brown, G.L.: The role of plasma amine oxidase, platelet monoamine oxidase, and red cell catechol-O-methyl transferase in severe behavioral reactions to disulfiram. Am. J. Psychiatry 136: 679-684, 1979.

Major, L.F., Lerner, P., Ballenger, J.C., Brown, G.L., Goodwin, F.K., and Lovenberg, W.: Dopamine-β-hydroxylase in the cerebrospinal fluid: relationship to disulfiram-induced psychosis. Biol. Psychiatry 14: 337-344, 1979.

Goyer, P.F., and Major, L.F.: Hepatotoxicity in disulfiram-treated patients. J. Stud. Alcohol 40: 133-137, 1979.

Goyer, P.F., Major, L.F., and Brown, G.L.: Alcoholism, aggression and depression. Am. J. Psychiatry (in press).

Lake, C.R., Ziegler, M.G., Major, F.L., Brown, G.L., and Ebert, M.H.: The effects of disulfiram on peripheral and central norepinephrine metabolism and blood pressure. In Fann, W.E., Haracan, I., Pohorny, A.D., and Williams, R.L. (Eds.): Phenomenology and Treatment of Alcoholism. New York, Spectrum Publications (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00334-01 CN

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of the pressor, metabolic and behavioral responses to intravenous tyramine.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

CO-PI:	David Pickar	Clinical Associate	CN NIMH
	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Robert M. Cohen	Clinical Associate	CN NIMH
	C. R. Lake	Staff Psychiatrist	LCS NIMH
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COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An increase in sensitivity to the pressor effects of tyramine, an indirectly acting sympathomimetic agent, is a well known side effect of MAO inhibitor treatment. Using an intravenous tyramine steady state infusion technique we are studying the effects of the selective inhibition of the A and B forms of MAO on tyramine's pressor effects in patients treated with clorgyline, pargyline and deprenyl. Our data suggest that treatment with clorgyline is associated with the greatest increases in tyramine sensitivity, while treatment with deprenyl or pargyline is associated with lesser increases in tyramine sensitivity. Changes in circulating norepinephrine accompanying the pressor changes induced by tyramine are being studied in unmedicated normal volunteers and in depressed patients before and during treatment with selective MAO inhibitors. Preliminary data has raised the possibility that a supersensitivity of the peripheral α-adrenergic receptor may contribute to the increased tyramine sensitivity observed during chronic MAO inhibition. We are also studying the effect of clinical state change in bipolar patients on tyramine response sensitivity, as well as the behavioral effects of tyramine-induced peripheral noradrenergic activation in patients and normal volunteers.

Project Description:

Objectives: The purpose of this project is to evaluate the comparative effects of selective MAO inhibition on the pressor response to tyramine and to study the biochemical and physiological effects of tyramine infusions in affectively ill psychiatric patients and in healthy normal volunteers. Since selective MAO inhibitors have proven useful in treating depression and hypertension, as well as adjuncts in the treatment of Parkinson's disease, an understanding of their effects on tyramine sensitivity is of great importance. As an indirectly acting sympathomimetic, tyramine is useful for the clinical evaluation of peripheral noradrenergic function. We are comparing the tyramine sensitivities of unmedicated patients with those of healthy normal volunteers. By measuring circulating catecholamines accompanying the physiological responses to tyramine, an indirect assessment of peripheral α-adrenergic receptor function can be achieved.

Methods Employed:

An intravenous steady state tyramine pressor response test is administered to drug free patients and again during the course of treatment with one of the following selective MAO inhibitors: clorgyline (a selective MAO A inhibitor), pargyline and deprenyl (selective MAO B inhibitors). The purpose is to achieve a 30 mm Hg rise in systolic blood pressure and to maintain this elevation for 5-10 minutes. Careful calculation of dosage and rates of infusion permit safe infusions both on and off the MAO inhibitors. That amount of tyramine per minute which maintains the pressor endpoint is the dose endpoint, and provides an index of tyramine pressor sensitivities for comparisons between subjects or treatment periods. Blood sampling is accomplished prior to and during the infusion. Samples are analyzed by enzyme isotope assay for catecholamines, by gas chromatography--mass spectroscopy techniques for MHPG, and by RIA assays for neuroendocrine parameters.

Major Findings:

We have found that clorgyline produces an approximately 30-fold increase in sensitivity to tyramine's pressor effects, while pargyline and deprenyl produce considerably lesser increases. Utilizing plasma MHPG as an index of MAO A activity and platelet MAO activity as an index of MAO B activity, our preliminary data suggests that the greater the inhibition of the MAO A enzyme, the greater the increase in sensitivity to tyramine. Differences in tyramine sensitivities produced by the selective B inhibitors may be attributable to loss of specificity (i.e., partial A form inhibition).

We have documented rises in circulating norepinephrine accompanying the tyramine-induced blood pressure elevations in both normal volunteers and patients. Chronic treatment with clorgyline and pargyline appear to decrease resting norepinephrine levels and may also dampen the rises of NE during the infusion, despite the achievement of similar pressor endpoints. These changes in the peripheral noradrenergic system suggest that MAO inhibiting antidepressants may alter regulatory mechanisms in addition to their selective MAO inhibiting effects.

The time course of the pharmacologic and biochemical changes brought about by the selective inhibitors are also under study, with particular reference to time course of the tyramine sensitivity changes. Preliminary findings suggest that the development of the greatly increased sensitivity to tyramine does not necessarily correspond in time to the onset of enzyme inhibition (monitored by plasma MHPG levels and inhibition of platelet MAO). Tyramine infusions after an acute MAO inhibiting dose of clorgyline, for example, after one week of clorgyline treatment and after four weeks of treatment show increasing pressor sensitivities. Continued longitudinal studies to focus on such time course issues are under way. The preliminary findings of decreased resting and pressor response-associated norepinephrine levels during chronic clorgyline treatment, and the longitudinal development of increasing pressor sensitivity beyond the time of initial enzyme inhibition, raise the possibility that a supersensitivity of the peripheral α-noradrenergic receptor contributes to the increase in tyramine sensitivity with MAO A inhibition.

Other investigators using a tyramine bolus injection technique have reported that depressed patients are significantly more sensitive to tyramine's pressor effects than normal controls. Using our steady state design our preliminary findings have not clearly supported these observations. We have found that affectively ill patients exhibit a wider range of tyramine sensitivities than normals, but do not appear to be overall more sensitive to tyramine.

In a detailed longitudinal study of a cycling bipolar patient, we observed changes in tyramine sensitivity during the course of the switch from depression to hypomania, raising speculation that such mood changes may involve changes in receptor sensitivity. We have also observed that tyramine induced pressor changes in two bipolar patients were associated with shifts from depression to hypomania which lasted as long as the blood pressure elevation, suggesting a possible vulnerability in central mechanisms to inputs from the peripheral noradrenergic systems. Evaluation of subjective and objective behavior changes induced by tyramine induced activation of the peripheral noradrenergic system in depressed patients and normal volunteers is currently in progress.

Publications:

Pickar, D., Cohen, R. M., Murphy, D. L., and Fried, D.: Tyramine infusions in bipolar illness: Longitudinal changes in pressor sensitivity, and behavioral effects. Am. J. Psychiatry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00335-01 CN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Smooth Pursuit Eye Tracking Impairment and Its Relation to Psychopathology and CNS Disorders.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. J. Siever	Clinical Associate	CN NIMH
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COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize psychological and psychobiological attributes that are associated with alterations in smooth pursuit eye movements. The smooth pursuit system regulates the eyes' smoothly following a moving target and can be measured by an electro-oculograph recording of an individual watching a moving pendulum. By using the high-risk strategy of screening a large group of college student normal volunteers, individuals with various alterations in smooth pursuit patterns will be identified. Individuals with two common types of alterations will be compared with those with more accurate tracking using a variety of psychological and biological tests to explore the relationship of these patterns to personality, psychophysiological and biochemical variables.

Project Description:

Objectives: This project is designed to study individual variations in smooth pursuit eye movements and their relationship to cognitive controls and personality tendencies. In order to understand such relationships, we are employing the "biological high risk strategy" i.e., identifying individuals from a population of normal volunteers with extreme values of a biologic variable implicated in the etiology of psychiatric disorders and comparing them psychologically with volunteers with normal values of that variable. This strategy has been employed successfully in studies of monoamine oxidase levels in man. College student normal volunteers will be screened to determine the quality of their smooth pursuit tracking. Subgroups of these students showing definite alterations in their smooth pursuit patterns will be compared to those with extremely accurate tracking on a variety of psychological, biochemical, and psychophysiological tests.

Methods Employed:

Volunteers from a local college, after giving informed consent, participate in a screening session testing their smooth pursuit eye movements. They are instructed to follow a moving pendulum, which makes a target excursion of 20 degrees of visual angle and oscillates at 0.4 HZ. Their eye movements are measured via surface electrodes by an electro-oculograph and both eye position and velocity are recorded on paper.

These records are scored for velocity arrests, i.e., points at which the eye stops moving while the target continues, and the pattern of deviation of the eye movements. For example, there are individuals with a high velocity arrest score and "spikey" pattern of eye movements as well as individuals with difficulty initiating eye tracking, so that saccades are substituted for the smooth pursuit movements, i.e., "saccadic substitution." A group of 20 individuals from each of these two groups will be compared with a matched control group exhibiting exceptionally good tracking patterns using a variety of psychological and biological tests as well as a clinical interview and family history.

Major Findings:

At this point, the project is midway through the screening phase. One hundred fifty volunteers have been screened for tracking pattern characteristics, including a number of individuals with high velocity arrest scores.

Significance to Biomedical Research and the Program of the Institute: The smooth pursuit system has been a focus of interest in psychiatry since it has been demonstrated that it is significantly impaired in psychotic individuals, particularly schizophrenics and some patients with severe affective disorders. This is hypothesized to represent a deficit in involuntary attention or cognitive centering. "Good" and "poor" tracking patterns have been shown to be under genetic control and this represents one of the few genetically determined biologic variables consistently associated with schizophrenic and other psychoses. By using the high-risk strategy of screening a college population for alterations in this system, the relationship of this variable to qualities of attention and personality tendencies in a non-psychiatrically defined population might be clarified without the confounding variables of the length of illness, medication, or hospitalization. This may facilitate delineating a dimension of psychological function closely tied to involuntary attention as reflected in this variable.

Proposed Course: We will be continuing to screen normal volunteers until we have sufficient numbers for each subgroup. Individuals selected on the basis of their tracking patterns will be contacted to come to the Clinical Center for further testing. This will include more elaborate testing of their smooth pursuit tracking in the Eye Clinic, biochemical testing for levels of monoamine oxidase and dopamine-β-hydroxylase in plasma, psychological testing including the MMPI, Rorschach and various attentional and cognitive tests; psychophysiologic testing including average evoked response and reaction time; neurological examination; a psychiatric interview, including a standardized format for research diagnosis; and a careful family history.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00446-10 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Inpatient Clinical Studies of Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Steven Paul	Clinical Associate	CP/NIMH
	Helena Calil	Guest Worker	CP/NIMH
	Robert Post	Chief, Clinical Res. Unit	BPB/NIMH
	Dennis Murphy	Chief,	CN/NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Unit, NIMH; Clinical Neuropharmacology, NIMH; St. Elizabeths Hospital; University of North Carolina (see supplemental sheet)

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.80	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of manic-depressive disorders, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are longitudinal; drug trials are double-blind. Major projects involve: (1) evaluation of tricyclic antidepressants that show specificity for a given monoamine neurotransmitter; (2) the study of phase shifts in sleep in cycling patients; (3) manipulation of sleep as a possible treatment of depression; (4) clinical and biochemical evaluation of electroconvulsive shock (ECT) in patients who have failed to respond to extensive and rigorous medication trials; (5) use of biological predictors of drug response including AER, MHPG, CSF, amine metabolites, neuroendocrine parameters and melatonin in blood and urine.

PHS-6040
(Rev. 10-76)

Project Description:

This description encompasses a wide range of research studies not covered in other individual project descriptions, many of which are continuations of ongoing investigations. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of manic-depressive and schizo-affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups, the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Work on the standardization of clinical methodology has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task a subjective "typicality" scale is assessed and an extended record of endogenous features are maintained.

As a contribution to ongoing efforts within the Division to standardize rating instruments across all four psychiatric wards, the Branch recently recruited Dr. Siewers from the Karolinska Institute in Stockholm, Sweden. Dr. Siewers, who played a leading role in the development of the C.P.R. rating system now in widespread use in Europe, has undertaken cross ward studies using a single rating team of multiple rating instruments.

In order to minimize uncontrolled environmental variables the ward milieu is structured according to a group model. Formal group psychotherapy is scheduled twice a week with a therapist and the head nurse. The only changes in group membership involve admissions or discharges of patients. A primary nurse and psychiatrist work together with each patient to provide medical care, to assist in decision-making, and to address concerns that arise. The goal of the ward structure is to provide the necessary optimal management for each patient. Although a comprehensive research program on the interaction of affective illness and the psychosocial milieu is not a part of the Branch's program, some specific questions in this area are addressed.

Behavioral data used in evaluating changes are collected on a longitudinal basis throughout the course of hospitalization using twice daily global ratings by a trained nursing research team; these ratings focus on depression, mania, psychosis, anger and anxiety. Twice a week a modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Information regarding families is obtained through ongoing contact between the social worker and the patient's relatives. Motor activity is recorded continuously as described in Project No. Z01 MH 00450-05 CP.

Sleep is recorded at half-hour intervals throughout the night on all patients. Selected patients have been studied for sleep parameters using all night EEG sleep recordings in collaboration with the central sleep facility of the DCBR (Dr. Gillin). This effort has expanded due to findings during the last year that sleep phases may systematically change in cycling patients and that manipulation of the time of day when an individual sleeps may provide a form of treatment (see Z01 MH 00450-05 CP). Certain patients may have sleep EEG's taken routinely for as long as a month.

All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness (see Project No. Z01 MH 01850-02 CP on Clinical Pharmacology).

The major areas of study and major findings during the last year can be divided into separate headings:

1. The main antidepressant drug trial underway on the ward involves a crossover between zimelidine and desmethylimipramine, the former having highly selective effects on reuptake in brain serotonin systems, while the latter is highly specific for norepinephrine systems (see Project No. Z01 MH 01850-02 CP.

2. In collaboration with Dr. Donnelly of SEH, NIMH and Dr. Murphy of the Clinical Neuropharmacology Branch, NIMH, several studies of psychological functioning in depressed and manic patients have continued. These studies include the Halstead-Reitan battery, a test of associative capacity thought to be a sensitive indicator of organic dysfunction, the Wechsler Adult Intelligence Scale, and the MMPI. Further results confirm previous preliminary findings that depressed patients show significant deficits on the Halstead-Reitan; in the case of the bipolar patients this deficit does not entirely return to normal during the immediate in-hospital recovery phase. Studies of IQ have indicated normal ranges for manic-depressive patients, but a striking change (averaging 20 points on the full scale) in the same patient going from depression into hypomania.

3. In collaboration with Monte Buchsbaum of the Section on Cognitive and Perceptual Studies, BPB, we have continued and extended our studies on the cortical evoked potentials in manic-depressive patients. This year a major focus has been on laterality in the evoked response. For details of these studies, see the Project report from the Section on Cognitive and Perceptual Studies, Biological Psychiatry Branch.

4. This year we undertook an analysis of the clinical results of ECT in a group of 13 patients who had received that treatment after failing to respond to extensive and rigorous treatment with psychotropic medications. All 13 of the patients showed a striking response to ECT with all of them experiencing virtually full recovery without complications.

Concurrent studies of CSF amine metabolites indicated that the principal effect of ECT treatment was a "normalization" of both the dopamine metabolite

(HVA) and the serotonin metabolite (5HIAA), that is the highest values were decreased while the lowest values increased.

In connection with each of the studies mentioned above our Branch focuses on a series of biochemical studies in patients described in detail elsewhere (indicated in parentheses):

a) Variations in plasma and urinary melatonin (Z01 MH 01851-02 CP)
b) Urinary excretion of MHPG as an index of norepinephrine turnover (combined central and peripheral) (Z01 MH 00448-06 CP)
c) Spinal fluid amines and amine metabolites (Z01 MH 00447-10 CP)
d) Multiple neuroendocrine measures, including response to specific challenge tests, in plasma, urine and CSF (Z01 MH 00452-04 CP)

All biochemical parameters are compared across clinical state, diagnostic subgroup, variables of age, sex and family history, as well as drug versus non-drug conditions and drug response.

Publications:

Ballenger, J.C., Goodwin, F.K., Major, F.L. and Brown, G.L.: Alcohol and central serotonin metabolism in man. Arch. Gen. Psychiat. 36: 224-227, 1979.

Goodwin, F.K. and Extein, I.L.: The biological basis of affective disorders. In Cancro, R., Shapiro, L. and Kesselman, M. (Eds.): Progress in the Functional Psychoses. New York, Spectrum Publications, 1979, pp. 129-152.

Donnelly, E.F., Murphy, D.L., Waldman, I.N. and Goodwin, F.K.: Prediction of antidepressant responses to imipramine. Neuropsychobiol. 5: 94-102, 1979.

Goodwin, F.K. and Zis, A.P.: Lithium in the treatment of mania. Arch. Gen. Psychiat. 36: 840-844, 1979.

Lipton, M.A., Carlsson, A., Dunner, D.L., Goodwin, F.K., Janowsky, D.S., Meltzer, H.Y. and Wyatt, R.J.: Biochemical and pharmacological Predictors. In Akiskal, H.S. and Webb, W.L. (Eds.): Psychiatric Diagnosis: Exploration of Biological Predictors. New York, Spectrum Publications, 1978, pp. 337-362.

Goodwin, F.K., Extein, I.L. and Wehr, T.A.: Some recent advances in psychobiology and psychopharmacology of affective disorders. In Ayd, F.J. and Taylor, I.J. (Eds.): Mood Disorders: The World's Major Public Health Problem. Baltimore, Ayd Medical Communications, 1978, pp. 22-43.

Gold, P.W., Goodwin, F.K., Davenport, Y.B. and Wehr, T.A.: The impact of normal volunteers on a research ward. Am. J. Psychiat. 136: 401-405, 1979.

Goodwin, F.K.: The crisis in psychiatric research. In Ayd, F.K., Jr. (Ed.): Crises in American Psychiatry. Baltimore, Ayd Medical Communications, in press.

Zis, A.P. and Goodwin, F.K.: Behavioral effects of L-DOPA in man. In Shader, R.I. (Ed.): Psychiatric Complications of Medical Drugs. New York, Raven Press, in press.

Lewy, A.J. and Goodwin, F.K.: Mental effects of reserpine in man: a review. In Shader, R.I. (Ed.): Psychiatric Complications of Medical Drugs. New York, Raven Press, in press.

Zis, A.P., Cowdry, R.W., Wehr, T.A., Muscettola, G. and Goodwin, F.K.: Tricyclic induced mania and MHPG excretion. Psych. Res. (in press).

Zis, A.P. and Goodwin, F.K.: Novel antidepressants and the biogenic amine hypothesis of depression: the case for iprindole and mianserin. Arch. Gen. Psychiat. (in press).

Goodwin, F.K. and Zis, A.P.: Lithium in mania: comparisons with neuroleptics and the issue of specificity. In Cooper, T., Gershon, S., Kline, N.S. and Schou, M. (Eds.): Lithium: Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00450-05 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biological Rhythms in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Philip Gold	Chief, Unit on Neuroendocrinol.	CP/NIMH
	Anna Wirz-Justice	Guest Worker	CP/NIMH
	Theodore Colburn	Chief	STD/NIMH
	Bruce Smith	Biomedical Engineer	STD/NIMH
	William Vaughan	Computer Programmer	STD/NIMH
	Howard Hoffman	Statistician	BB/NICHD
	J. Christian Gillin	Chief, Unit on Sleep Studies	BPB/NIMH
	Elliot Weitzman	Chairman, Dept. of Neurology	Albert Einstein Med. Sch.

COOPERATING UNITS (if any)
Section on Technical Development, NIMH; Biometry Branch, NICHD; Unit on Sleep Studies, Biological Psychiatry Branch, NIMH; Albert Einstein Medical School, New York

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.40	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Basic research in biological rhythms can be related to affective illness with respect to (1) the inherent cyclicity of the illness (itself a type of biological rhythm) and (2) the involvement of disturbed circadian rhythms (24-hour cycles) in its pathophysiology. This project is designed to explore these connections by interrelating behavioral, physiological and biochemical changes observed (1) through the cycle of the illness, and (2) through the course of the 24-hour day. Results to date indicate (1) that in a subgroup of female bipolar patients the natural rate or frequency of cycling between mania and depression is accelerated 4-fold by maintenance tricyclic antidepressants. (2) Tricyclics also accelerate the frequency of free-running circadian activity rhythms in hamsters studied in our laboratory. (3) Depressive and manic patients show state-dependent phase shifts (changes in timing) of circadian rhythms in temperature, motor activity, sleep architecture and neurotransmitter metabolites; in this circadian desynchronization, the rhythm is not "locked in" to the environmental 24-hour day-night cycle, but tends to drift in and out of phase. (4) In phase shift experiments it has been possible to switch patients out of depression by directly manipulating the timing of circadian rhythms by changing patients' sleep schedules.

PHS-6040
(Rev. 10-76)

Project Description:

Epidemiological evidence has emphasized the essential cyclicity of both unipolar and bipolar affective illness. Since manic-depressive illness is a periodic illness, a study of the role of biological rhythms in its pathophysiology and pathogenesis is important. This project involves longitudinal studies of behavioral, physiological, and biochemical variables in inpatients and outpatients who have relatively frequent mood cycles. Focus is on both circadian (i.e., 24-hour) and longer rhythms, requiring both intensive data collection (multiple sampling within the same day) and longitudinal data collection over time (in several patients, we have obtained daily measures for a period of many months).

Behavioral parameters studied involve both nurses' ratings and self-ratings, as well as motor activity monitoring using a novel nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies, temperature, biochemical variables including urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the urinary metabolite thought best to reflect brain norepinephrine metabolism, melatonin, cortisol, thyroid stimulating hormone (TSH), growth hormone (GH), prolactin (PL), and (less frequently) CSF amine metabolites. These data are evaluated by time series analyses in order to characterize circadian and longer rhythms.

We can describe the findings in these studies under several headings:

1. We had previously identified a group of bipolar cycling patients who show circadian rhythm desynchronization in temperature and in motor activity and have now extended the findings to additional patients. In these patients, circadian rhythms are not locked into phase with the environmental day-night cycle, but tend to drift gradually in and out of phase. In this respect, these patients behave as if they had been cut off from all environmental light and time cues; these findings suggest some disconnectedness in the systems responsible for integrating environmental cues into the regulation of biological rhythms. The systems involved in this integration include hypothalamic clock mechanisms, the pineal gland and connecting central norepinephrine pathways, and will be a focus of further study in the coming year.

2. We have extended and completed a study designed to compare diurnal patterns of urinary MHPG excretion using continuous three-hour urine collection periods in depressed patients and normal controls. We found a diurnal pattern for urinary MHPG in normal controls with a maximum in the daytime and a minimum at night. Depressed patients as a group show an alteration in timing of diurnal MHPG variation (an earlier peak) as well as amount (lower mean). Compared with controls, depressed patients show decreases in MHPG that are maximal in the late evening. This finding is consistent with the hypothesis that diminished central noradrenergic activity is responsible for abnormal cortisol secretion and decreased REM sleep latency in the late evening reported to exist in depressed patients, and that the timing or phase of circadian rhythms is shifted ahead in depressed patients.

3. Continuous monitoring of motor activity has become a principal focus of ongoing longitudinal studies of affective disorder. Activity is measured with a small, ambulatory solid state device worn on the wrist. Data are periodically retrieved and analyzed by computer. Continuous monitoring each 15 min. makes it possible to measure the amount and, equally important, the timing of activity in patients as clinical state changes.

The level of motor activity and the temporal distribution of activity, as other physiological functions, is regulated by neural mechanisms in the central nervous system with known input from noradrenergic, serotonergic, dopaminergic, and cholinergic neurotransmitter systems. In particular, the 24-hour activity-rest cycle is driven by a hypothalamic "pacemaker" in animals and has been the focus of intensive investigation in basic biological rhythm research. Recurrent depressive and manic-depressive illness may be conceptualized as disorders of this activity-rest cycle "pacemaker" in as much as activity monitoring has revealed: a) consistent state-dependent changes in amount of activity; b) changes in timing (phase shifts or frequency modulation) of the daily onset of activity; c) state-dependent changes in the ratio of the length of the daily activity phase to the length of the daily rest phase. Ten patients have been monitored through one or more complete manic-depressive cycles. Eight of these experienced 48-hour or bicircadian sleep-wake cycles at the switch into mania. Our review of nurses' sleep checks in 62 manic patients indicate at least 56% had 48-hour sleep-wake cycles during manic episodes. This phenomenon is known to occur spontaneously in normal subjects isolated from external time cues. These findings provide further support for the hypothesis that affective patients fail to respond normally to external time cues.

It is of particular importance that each of these findings can be induced experimentally in animals with environmental (light, schedules), chemical (endocrine, drug) and anatomical (hypothalamic lesions) manipulations. Thus, motor activity monitoring provides a direct bridge between disturbed behavior in psychiatric patients and experimentally-induced behavior in animals, and relates this behavior to central neuroregulatory systems that have been intensively investigated in animals for many years.

Using the existing NIMH activity monitor, we have extended our studies of cyclic affective disorders to several additional patients, some of whom show state-dependent circadian rhythm desynchronization. To further our understanding of mechanisms that regulate circadian activity rhythms, we have undertaken to study longitudinally (1) primary circadian rhythm disturbances in non-affectively ill patients such as insomniacs (one such patient who is unable to adhere to a 24-hour schedule and lives on a 25-hour "day" has been intensively studied for one year). Of special interest are patients with difficulty falling asleep and waking up due to phase-delay of circadian rhythms. These patients are being studied in collaboration with Dr. J. C. Gillin, Chief, Unit on Sleep Studies, BPB. (2) and normal humans who are free-running and paced entirely by their internal clocks in complete isolation from environmental time cues (in collaboration with Elliot Weitzman at the Laboratory of Human Chronophysiology, Albert Einstein Medical School, New York).

Monitoring of subjects for periods exceeding one year has made it essentia that we develop techniques of data reduction and analysis. Major progress in this area has been made during the past two years. In collaboration with William Vaughn of the Technical Development Section, we have developed a series of interactive computer programs designed to display and analyze large amounts of activity data rapidly. For example, using graphic techniques ("actograms") that are universally accepted in the representation of longitudinal animal activity data, it is now possible to plot a year's continuous activity in a single diagram and thus demonstrate trends in parameters of the activity rest cycle. Algorhythms that detect artifacts, determine length of sleep, times of awakenings, changes in levels of activity and distribution of activity throughout the day have also been developed. Changes in these variables are clinically relevant and it is expected that the instrumentation together with the automatec data processing package may become useful and cost-effective in large scale clinical and investigational assessment of patients concerning diagnosis, drug response and course of illness.

Limited data-storage capacity of the present monitor that requires three times a week computer "read-out" has usually made outpatient monitoring impractical. A major development this year has been the design of 50 new monitoring units with extended electronic memory requiring only weekly "read-out". Unfortunately, implementation of clinical outpatient studies has not begun, because the manufacturer who was awarded the contract for the production of these units (following S.B.A. guidelines) has not yet met his February 4, 1979 deadline. Nevertheless, large scale outpatient studies are anticipated in the coming year. Activity monitoring will prove to be the only continuous clinical data base for outpatient studies in the new ACRF.

In order to exploit the potential of motor activity monitoring as a behavioral bridge between psychiatric patients and experimental animals, we have constructed an animal laboratory designed (with Theodore Colburn and his associates in the Technical Development Section) to monitor longitudinally the circadian activity-rest cycle in 16 hamsters under constant environmental conditions. A microprocessor continuously monitors wheel-running activity and stores data on magnetic tape. The data can be analyzed and plotted using the same programs and format that have been developed for human studies and thus, can be directly compared with human data. With this facility, in operation for 10 months, it has been possible to test the effects of chronically administered psychoactive drugs on the mechanisms that regulate circadian rhythms in these "free-running" animals. Lithium ion is already known to alter the intrinsic rhythm or frequency of the hypothalamic circadian pacemaker. We have found that tricyclic antidepressants that alter the timing or phase of circadian rhythms in some affective patients during clinical response to the drug, and which induce rapid cycling in some patients, also accelerate the intrinsic periodicity of the free-running hypothalamic clock in experimental animals. Currently, the second major class of antidepressants, monoamine oxidase inhibitors, is being tested.

4. The transient antidepressant effect of sleep deprivation therapy has been thought to be mediated by mechanisms that regulate the synchronization of

circadian rhythms with the environment. We have recently tested this hypothesis with an experiment specifically designed to manipulate the timing or phase of circadian rhythms without decreasing sleep. Reasoning that sleep deprivation in man is equivalent to continuous light in animals, one would predict on the basis of animal experiments that continuous wakefulness induces a phase advance in circadian rhythms, along with increased overall activation (according to "Aschoff's Rule"). This hypothesis predicts that advancing a depressed patient's daily schedule several hours earlier than usual would, as sleep deprivation, cause a similar phase shift in circadian rhythms accompanied by increased activation. Sleep deprivation has only a transient (one day) antidepressant effect. In contrast to one night's sleep deprivation, however, the phase advance experiment can be sustained for many days and would be expected to have a sustained antidepressant effect. A depressed patient who had consistently and dramatically responded to one night's sleep deprivation was placed on a six hour phase advanced daily schedule. She responded with a dramatic and sustained remission. Three other depressed patients have been screened for similar phase shift experiments using the response to one night's total sleep deprivation as a predictor of response to circadian phase shifting. In two cases, the response to phase shifting was consistent with the response to sleep deprivation (partial improvement). Future phase shift experiments will include EEG sleep monitoring; existing studies indicate that percentage REM and REM latency can be systematically altered by transmeridianal flight. Changes in these parameters would be expected to occur during phase shift experiments and would be of interest in view of numerous studies that show state-dependent changes in these variables associated with cyclic mood disorders.

5. One of our efforts to characterize circadian rhythm alterations in affective disorders uses frequent sampling of concentrations of blood constituents obtained via an indwelling catheter. Studies of circadian neuroendocrine profiles during mania and depression in the same, unmedicated patients have been completed for six bipolar patients to date, and samples are being assayed for GH, FSH, LH, TSH, PL and cortisol by Dr. Gregory Brown in Toronto.

6. In collaboration with Dr. Post of the BPB, we have analyzed the CSF metabolite data from the last 10 years for seasonal rhythms. The variations in the baseline values did not show any consistent seasonal pattern. However the data on probenecid induced accumulation of metabolites showed seasonal variation: CSF HVA and 5HIAA showed a trough in spring and a peak in autumn, while MHPG had the opposite seasonal rhythm. Unexpectedly, analysis of the CSF probenecid concentrations themselves yielded an annual rhythm with a nadir in spring and the highest values in summer. This finding raises serious methodological issues, since all patients were given the same dose/body weight. An investigation on a larger sample of normal subjects (by the National Center for Health Statistics), using serum urates as an indicator of blood-brain-CSF transport characteristics was started. Such an independent study will show whether the fluctuation in CSF probenecid values in spite of constant dosage is related to seasonal changes in transport mechanisms.

Together with Dr. Ray Lake (LCN) we are similarly reanalyzing for seasonality his large collection of serum and CSF and DBH data. A preliminary analysis showed an annual rhythm of large amplitude, with a minimum in summer and a maximum 60% increase in winter.

7. Our earlier observations that antidepressant medications alter the time course of recurrent affective disorders have now been extended to a larger group of patients (n=9). Tricyclics have markedly accelerated mood cycle frequency from one cycle every few months to one cycle every few weeks or days in a subgroup of female manic-depressive patients. Therefore, these drugs seem to act by driving rather than counteracting the pathophysiological process underlying recurrent depressive illness. As mentioned previously, this process may involve changes in timing of circadian rhythms. This hypothesis will be explored in animal studies; exploring the effects of antidepressants on the spontaneous (free-running) frequency of hypothalamic biological oscillators that drive the activity-rest cycle.

8. The observation of rapid cycling induced by maintenance tricyclic antidepressants in manic-depressive patients, as well as the speeding up of circadian rest-activity rhythms in the behavioral model of the hamster, led us to consider linking these findings to biochemical mechanisms. To date the indication of beta-adrenergic receptor sub-sensitivity is the most consistent finding of the common mode of action of chronic antidepressants. The effect of chronic antidepressants on alpha-adrenergic receptor sensitivity is unclear, since reports are conflicting. Therefore in collaboration with Marion Kafka of the BPB, Dr. Anna Wirz-Justice undertook circadian rhythm studies of adrenergic receptor sensitivity in the brain, as well as the effect of chronic antidepressant treatment on this rhythm. We found a clear and reproducible rhythm in the number of alpha- and beta-adrenergic receptors in the CNS, which showed differences in timing of the peaks and troughs. Chronic treatment with imipramine resulted in a marked 12-hour phase shift in the alpha-adrenergic rhythm, as well as a slight decrease in the 24-hour mean. In the beta-adrenergic rhythm there was a slight 4-hour phase shift, together with a marked decrease in the 24-hour mean, without an amplitude change.

The circadian rhythm of dopamine receptors in the striatum was also studied. Here a bimodal rhythm was found, with peaks in the mid-light and in the mid-dark phase. The rhythm was more pronounced than that of the alpha-and beta-receptors, being of the order of 100% increases compared with 30-40%. The effect of chronic imipramine on this rhythm was less pronounced, with clear reduction of DA receptors only at the peak at 2 a.m. These results on circadian rhythms in neurotransmitter receptors and their modification by drugs open up a new direction of research into physiological regulatory mechanisms occurring over very brief time periods.

9. Working in Dr. Markey's lab, Dr. Lewy has developed a gas chromatographic-mass spectroscropic (GC-MS) assay for melatonin. Melatonin, produced by the pineal gland, is thought to be an indicator, if not a mediator, of a central "biological clock", and is thus another critical methodological underpinning for the studies of biological rhythms (see Project No. Z01 MH 01851-02 CP).

Publications:

Wehr, T.A. and Goodwin, F.K.: Rapid cycling in manic-depressives induced by tricyclic antidepressants. Arch. Gen. Psychiat. 36(5): 555-559, 1979.

Wehr, T.A. and Goodwin, F.K.: Biological rhythms and affective illness. In Flach, F.F. (Ed.): Weekly Psychiatry Update Series. New York, Biomedia, Inc., 1978, Vol. II, pp. 2-7.

Wehr, T.A. and Goodwin, F.K.: Tricyclic antidepressants accelerate manic-depressive cycles. Chronobiologia (in press).

Wehr, T.A. and Goodwin, F.K.: Biological rhythm disturbances in affective illness. Proceedings of the Second World Congress of Biological Psychiatry. Amsterdam, Elseview/North Holland (in press).

Wehr, T.A., Wirz-Justice, A., Duncan, W., Gillin, J.C., and Goodwin, F.K.: Phase advance of the circadian sleep-wake cycle as an antidepressant. Science (in press).

Wehr, T.A., Wirz-Justice, A., and Goodwin, F.K.: Tricyclics shorten hamster circadian period. Chronobiologia (in press).

Wehr, T.A. and Goodwin, F.K.: Circadian rhythm desynchronization as a basis for manic-depressive cycles. Psychopharm. Bull. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01851-02 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Melatonin Studies in Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	William Potter	Staff Psychiatrist	CP/NIMH
	Sanford Markey	Chief, Pharm. Appl. of Mass Spec.	LCS/NIMH
	Irwin J. Kopin	Chief	LCS/NIMH
	Wallace Mendelson	Staff Psychiatrist	BPB/NIMH
	Lawrance Tamarkin	Staff Fellow	OD/NICHD
	Neal Cutler	Clinical Associate	BPB/NIMH
	David Newsome	Staff Ophthalmologist	CB/NEI

COOPERATING UNITS (if any)
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NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.98	

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The role of the pineal gland and its hormone, melatonin, has been difficult to define in humans, because the circulating levels of melatonin have been too low to measure. Recently, in collaboration with Dr. S. Markey of the LCS, we have developed a highly sensitive and specific (GC-MS) assay which requires less than 1 ml of human plasma, CSF, or urine. Since the development of this assay, melatonin research has been extended into two broad areas: (1) investigation of the pattern of secretion of melatonin as affected by circadian, seasonal, and menstrual rhythm; the effect of light, drugs, and sleep; and in various pathologic syndromes (precocious puberty, delayed onset of puberty, infertility, hypothalamic, pituitary, adrenal, and thyroid diseases, hypertension, breast and ovarian malignancies, as well as affective disorders and schizophrenia); (2) investigations of this hormone as a "marker" for (a) the biologic "clock", and (b) β-adrenergic receptor function. We have found that sunlight, but not artificial light of normal intensity suppresses melatonin secretion in humans, that melatonin is increased in mania and decreased in depression, that chronic tricyclic antidepressant treatment reduces melatonin, and that propranolol (a β-adrenergic antagonist) abolishes melatonin secretion.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The pineal gland is of considerable interest not only because its basic role in endocrine function in man is unknown, but also because it provides an *in vivo* marker for the biological clock regulating the activity-rest cycle (see below), as well as being a measure of the activity of a well-characterized beta-adrenergic receptor. In man plasma levels of melatonin are extremely low, on the order of 1-2 picogram/ml during the day, and have not been possible to measure by previous techniques. Dr. Lewy has developed a gas chromatographic-mass spectrometric (GC-MS) method for the measurement of melatonin in collaboration with Dr. Sanford P. Markey of the LCS. This technique has the requisite specificity and accuracy to measure melatonin in human plasma in quantities as little as 1 cc of plasma. In addition to affective illness, other diseases of interest include those involving the thyroid and adrenal, precocious and delayed onset of puberty, infertility, hypertension, and malignancy, including cancer of the breast and ovary.

The neurotransmitter norepinephrine is the normal physiologic stimulant of the pineal gland's secretion of melatonin. Attention has been focused on this neurotransmitter in conjunction with affective disorders and other psychiatric diseases. We are studying the efffects of various psychotropic medications, including tricyclic antidepressants, lithium, neuroleptics, and MAO inhibitors, both acutely and chronically. Many of these medications have been thought to induce changes in β-adrenergic receptors. It is now possible to document *in vivo* these changes in humans.

Methods: Although radioimmunoassays (RIAs) and bioassays were used for measurement of human plasma melatonin, problems with insufficient sensitivity, specificity or efficiency have severely limited their use for routine analysis. Whereas theoretically GC-MS is the most specific of these techniques, attempts to apply it to melatonin analysis have also failed because of insufficient sensitivity. Use of a new technique, negative chemical ionization, and improved chromatographic and extraction procedures, lead to a GC-MS method that has the requisite sensitivity, specificity and efficiency for the routine analysis of melatonin in human plasma, CSF and urine. Less than 1 ml of sample is required, thus, permitting wide flexibility of experimental design and repeated and frequent measurements. Daytime concentrations as low as 1-2 picogram/ml and nighttime values as high as 100 picograms/ml can be measured, yielding a 20-50 fold range. The other available assays (RIAs and bioassays) generally report higher values and a lower range, probably because of their lesser specificity.

Major Findings: Humans display the predicted nighttime rise and daytime fall of melatonin secretion, as do other subhuman species. Normal controls secrete melatonin to a maximum 25-100 picograms/cc during the night. Manic patients, however, secrete significantly more melatonin during the night than do normals or depressed patients. Bipolar depressed patients secrete less melatonin than do normals or manic patients. These results are consistent with the catecholamine hypothesis of affective disorder, which states that noradrenergic activity is increased in mania and decreased in depression.

We have also determined that artificial light of normal intensity does not affect melatonin secretion in man. In animals, artificial light of any intensity blocks melatonin secretion abruptly and completely. However, humans will suppress under artificial light of the approximate intensity of sunlight. In other words, humans have adapted or evolved to distinguish between artificial light of normal intensity and sunlight, so that their biological rhythms are not synchronized by the changes in artificial lighting during the day, but only by dusk and dawn.

In collaboration with Dr. W. Mendelson of the Sleep Lab (BPP), we studied the stages of sleep in relation to melatonin secretion. There appears to be a close correlation between bursts of melatonin during the night, and various stages of sleep. Animals studies include the effect of melatonin and propranolol on rat sleep. The effects of melatonin and propranolol seem to be opposite, depending on whether or not the species is nocturnal or diurnal.

We are interested in the effect of propranolol, the β-adrenergic receptor blocker, in the treatment of manic-depressive illness. Uncontrolled studies of propranolol in various psychiatric disorders suggest positive effects in states associated with hyperactivity, such as mania and agitated depression. We are extending these studies using a double-blind paradigm, and our preliminary findings are encouraging. By following plasma melatonin, it is possible to determine the relationship between clinical effects and beta blockade. We note a precise dose-response relationship between the amount of propranolol and the blockade of melatonin secretion at night.

Using the melatonin assay, we have studied the effects of the tricyclic antidepressant DMI on noradrenergic function in animals. Overall output of the noradrenergic system controlling melatonin secretion can be assessed by measuring the hormone at night. Changes in postsynaptic receptor function can be delineated by measuring the response of melatonin to isoproterenol injection during the day. The state of presynaptic function can therefore be estimated by subtraction. We have found that DMI causes a 20% reduction in isoproterenol stimulation of melatonin during the day, implying a 20% reduction in postsynaptic β-adrenergic receptor function. However, the nighttime melatonin secretion is reduced by more than 50%, indicating that some reduction in presynaptic function is also associated with DMI treatment.

Discussion: The methodology for developing melatonin has been the "bottleneck" for the last several years in preventing elucidation of the role of the pineal gland in humans. Moreover, melatonin's utility as a marker for the biologic clock and for β-receptor sensitivity has been hampered by the lack of an accurate and specific melatonin assay. After three years of development, it is now possible to use this assay in both laboratory and clinical research. Many projects are in progress now, and many will be initiated during the next year. For example, we will be collaborating with the National Eye Institute with Dr. Elmer Ballentine and Dr. David Newsome. We will be looking at melatonin levels in patients with retinitis pigmentosa. This disease has no known cause, and because of the link with a pigmenting agent such as melatonin, it is possible that the explanation for this disease may be in understanding melatonin levels. We will also be studying the melatonin diurnal patterns of secretion, in blind patients. Changes in melatonin secretion may help to explain a number of associated disorders in blind people, such as sleep and mood disorders, and infertility. A major collaboration within the branch with Dr. Thomas Wehr (see Report No. Z01 MH 00450-05 CP) will be continued, examining the melatonin levels in patients with disturbed biological rhythms and patients with sleep disorders.

We will also be collaborating with Dr. Irwin Kopin in a study of hypertension and the role of the β-adrenergic receptor in hypertensive patients and in genetically hypertensive rats. In addition, we will be looking at melatonin levels in various neurologic disorders, including Shy-Draeger syndrome. These patients are thought to have minimal β-adrenergic tone.

The effect of light on melatonin may open an entirely new area of research. We will be elucidating the dose-response curve of light intensity and wavelength. Using light as a nonpharmacologic tool we are better prepared to examine the relationship between the pineal gland, the biologic "clock" in the suprachiasmatic nucleus.

Publications:

Lewy, A.J. and Markey, S.P.: Analysis of melatonin in human plasma by gas chromatography negative cehmical ionization mass spectrometry. Science 201: 741-743, 1978.

Markey, S.P., Lewy, A.J., and Colburn, R.W.: Studies in biogenic amine metabolism by mass spectrometry. Proceedings of the International Symposium on Quantitative Mass Spectrometry in the Life Sciences. Amsterday, Elsevier, 1978, pp. 17-37.

Markey, S.P. and Lewy, A.J.: Quantitative analysis of melatonin in human plasma by negative chemical ionization mass spectrometry. Proc. Ninth Materials Research Symposium. National Bureau of Standards, Special Publication 519, Trace Organic Analysis: A New Frontier in Analytical Chemistry, 1979, pp. 399-404.

Lewy, A.J., Wehr, T.A., and Goodwin, F.K.: Melatonin secretion in manic-depressive illness. Proceedings of the Second World Congress of Biological Psychiatry. Amsterday, Elsevier/North Holland (in press).

Wehr, T.A., Wirz-Justice, A., Lewy, A.J., and Goodwin, F.K.: Biological rhythms in affective illness. Proceedings of the Second World Congress of Biological Psychiatry. Amsterdam, Elsevier/North Holland (in press).

Lewy, A.J., Wehr, T.A., Gold, P.W., and Goodwin, F.K.: Plasma melatonin and manic-depressive illness. In Usdin, E., Kopin, I.J., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, Volume II, pp. 1173-1175.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00447-10 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cerebrospinal Fluid Amine Metabolites and Amines in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frederick Goodwin	Chief	CP/NIMH
OTHER:	Rex Cowdry	Staff Psychiatrist	CP/NIMH
	Irl Extein	Clinical Associate	CP/NIMH
	Thomas Wehr	Chief, Clinical Res. Unit	CP/NIMH
	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinol.	CP/NIMH
	William Potter	Staff Psychiatrist	CP/NIMH
	Robert Post	Chief, Clinical Res. Unit	BPB/NIMH
	Edna Gordon	Chief, Unit on Clinical Biochem.	LCS/NIMH
	Daniel van Kammen	Chief, Unit on Neuropsychopharm.	BPB/NIMH
	Francis Major	Clinical Associate	CN/NIMH
	Ray Lake	Staff Psychiatrist	LCS/NIMH
	Pauline Lerner	Staff Scientist	BPB/NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS: 1.25	PROFESSIONAL: .70	OTHER:

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Amine metabolites in cerebrospinal fluid (CSF) are studied in patients to assess neurochemical alterations that may be associated with clinical diagnoses (particularly affective illness, schizophrenia, personality disorders, and alcoholism), biological subgroups, or drug treatment. Probenecid, which inhibits the removal of the metabolites from CSF into the blood, is administered as part of these studies. Low pretreatment probenecid-induced levels of HVA are associated with a response to piribedil, a dopamine agonist. Lithium and ECT produce similar changes in amine metabolites: 5HIAA decreases significantly, while the HVA response depends on pretreatment HVA levels. The desmethylimipramine/zimelidine crossover trial demonstrates relative specificity of amine metabolite changes; the noradrenergic tricyclic selectively decreases CSF MHPG, while the serotonergic agent selectively decreases CSF 5HIAA. The dopamine agonist, piribedil, decreases CSF HVA during treatment. CSF calcium is positively associated with the severity of depression, decreases as depressed patients improve and increases as schizophrenic patients improve.

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Michael Ebert	Chief, Sec. on Exp. Therap.	LCS/NIMH
James Ballenger	Clinical Associate	BPB/NIMH
David Jimerson	Staff Psychiatrist	LCS/NIMH

Project Description:

The Chief, CPB, serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB provides sample processing and assays (the latter in collaboration with Edna Gordon of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Dr. Murphy).

This project explores the neurochemistry of various psychiatric conditions using clinical methods previously developed in this Branch, and lab techniques developed in the Branch and in the Unit on Clinical Biochemistry, LCS.

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients by assay of levels of the amines and their major metabolites in the cerebrospinal fluid (CSF) under baseline conditions and following the administration of probenecid. The metabolites under study are 5-hydroxyindoleacetic acid--5HIAA, the major metabolite of serotonin, homovanillic acid--HVA, the major metabolite of dopamine, 3-methoxy-4-hydroxyphenylglycol acid--MHPG, and 3-methoxy-4-hydroxyphenylacetic acid--VMA, the major and minor metabolites, respectively of norepinephrine; in addition, norepinephrine itself is measured in collaboration with Dr. Ray Lake of the LCS, and dopamine-β-hydroxylase--DBH is assayed in collaboration with Dr. Pauline Lerner of the BPB.

In collaboration with these units, we have continued the exploration of CSF amine metabolites within and between various diagnostic groups (affective illness, schizophrenia, and schizoaffective disorders) with the emphasis on primary affective illness. In addition, in collaboration with Dr. James Ballenger, we have studied amine metabolites in the spinal fluid of normal volunteers, relating the baseline values to a variety of personality variables. Thirty volunteers have now been studied. Finally, a collaboration with the National Navy Medical Center, completed last year, has enabled us to broaden our diagnostic subgroup studies to include a significant number of individuals with alcoholism and/or character disorder. By broadening our study populations, we hope both to develop control group values for comparison with other diagnostic groups and to identify features of personality or behavior that may be related to monoaminergic function across diagnostic groups.

One interesting finding differentiating diagnostic groups relates to the response to the procedure itself. Ballenger et. al. report that the incidence of post-lumbar puncture headaches is significantly higher in normal volunteers than it is in patients with primary affective disorder or schizophrenia, studied under the same conditions. One possible hypothesis suggests that pain thresholds may be altered in these psychiatric disorders.

While few other major diagnostic group differences have been demonstrated, the study of neurotransmitters, their metabolites, and their metabolizing enzymes within diagnostic groups has been fruitful. For example, in primary affective disorder, CSF norepinephrine (NE) is higher in mania than in depression; within the depressed patient group, CSF NE is higher in those with high anxiety (Post, et. al). These suggest a relationship between CSF NE and either psychic or motoric activation. Within a group of patients with alcoholism, Major et. al. found that low levels of CSF DBH were associated with a markedly elevated MMPI profile, with particularly significant elevations on scales Pd, Pt, and Sc; thus, since mean DBH is similar across diagnostic groups, DBH may be most closely related to certain personality features that are somewhat independent of diagnosis.

Similarly, Jimerson et. al. report no significant diagnostic group differences in CSF calcium. However, levels in depression are higher than those in mania in four rapid-cycling patients. Within the depressed group, calcium was positively correlated with symptom severity, and tended to decrease with improvement, whether spontaneous or lithium-related. Finally, within the group of schizophrenics, there was a significant increase in CSF calcium during symptomatic improvement. These findings may be related to changes in neuronal and physiological excitability associated with variations in calcium levels.

A series of reports from this group relating initial metabolite levels to treatment response have appeared over the last 10 years, suggesting that low pretreatment levels of a metabolite are associated with good antidepressant response to drugs whose predominant initial action is to enhance the activity of the neurotransmitter in question. Post et. al. published a study this past year that reports greater antidepressant response to piribedil (a dopamine receptor agonist) in patients with low pretreatment CSF levels of HVA (a dopamine metabolite).

In addition, this study reports that CSF HVA decreases during treatment with piribedil, a finding paralleling the effect of serotonergic antidepressants on CSF 5HIAA. These results are consistent with a negative feedback mechanism regulating neurotransmitter systems.

Two studies in process report striking similarities between CSF metabolite changes induced by ECT and those induced by lithium. Cowdry finds that lithium significantly decreases CSF 5HIAA, but that the effects on HVA depend on the pretreatment level of HVA; low initial levels increase during treatment, while high levels decrease. Extein finds an identical pattern of metabolite response to ECT.

Finally, the desmethylimipramine/zymelidine crossover study is nearing completion. Preliminary results suggest that their in vitro specificity for blocking noradrenaline reuptake and serotonin reuptake respectively is mirrored in relatively specific effects on CSF metabolites, with desmethylimipramine selectively decreasing CSF MHPG while zymelidine selectively decreases CSF 5HIAA.

Studies of levels of tricyclic antidepressants in CSF reveal that 1) variations in metabolism of imipramine account for almost all variation in the CSF concentrations of the drug and 2) the imipramine-induced alteration in CSF levels of 5HIAA correlated with CSF imipramine levels (a moderately serotonergic agent) but not with CSF desipramine levels (a metabolite with little serotonergic activity).

A series of methodological questions are under study. Jimerson has published a report suggesting that while fluorometric values of HVA are consistently lower than gas chromatographic/mass spectrometric (GC/MS) values, the results are highly correlated. Fluorometry is a satisfactory assay method, at least in the high ranges of HVA achieved with probenecid. However, we have placed increasing emphasis on baseline studies, partially as a result of the discovery of confounding sources of variance in the probenecid method. This necessitates increasing reliance on GC/MS assays done collaboratively by the LCS.

Current interests under investigation include the study of a variety of neuropeptides and hormones in CSF. A GC/MS assay for melatonin (see report Z01 MH 01851-01 CP) permits the reliable measurement of the minute quantities of melatonin found in CSF. Assays for vasopressin in CSF may provide an avenue for exploring hypotheses linking vasopressin with primary affective disorder. Finally, the availability of results from Dr. Gold's integrated neuroendocrine test will allow closer examination of the relationships between CSF metabolites and neuroendocrine challenge tests as markers of central neurotransmitter activity.

Publications:

Ballenger, J.C., Sternberg, D.E., Cowdry, R.W., Post, R.M., van Kammen, D.P., and Goodwin, F.K.: Post lumbar puncture headaches in psychiatric patients and normal volunteers: differential incidence (letter). NEJM (in press).

Brown, G.L., Goodwin, F.K., Ballenger, J.C., Goyer, P.F., and Major, L.F.: Human aggression and its relationship to cerebrospinal fluid 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenylglycol, and homovanillis acid. In Sandler, M. (Ed.): Psychopharmacology of Aggression. New York, Raven Press, 1979, pp. 131-148.

Cowdry, R.W., and Goodwin, F.K.: Biological and physiological predictors of drug response. In van Praag, Rafaelson, Lader and Sachar (Eds.): Handbook of Biological Psychiatry. New York, Marcel Dekker, Vol. IV (in press).

Ebert, M.H., Kartzinel, R., Cowdry, R.W., and Goodwin, F.K.: Cerebrospinal fluid amine metabolites and the probenecid test. In Wood, J. (Ed.): Neurobiology of Cerebrospinal Fluid. New York, Plenum Press (in press).

Jimerson, D.C., Gordon, E.K., Post, R.M., and Goodwin, F.K.: Homovanillic acid in human cerebrospinal fluid: comparison of fluorometry and gas chromatography-mass spectrometry. Comm. in Psychopharmacol. 2: 343-350, 1978.

Jimerson, D.C., Post, R.M., Carman, J.S., van Kammen, D.P., Wood, J.H., Goodwin, F.K., and Bunney, W.E., Jr.: CSF calcium: clinical correlates in affective illness and schizophrenia. Biol. Psychiat. 14: 37-53, 1979.

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Muscettola, G., Goodwin, F.K., Potter, W.Z., Claeys, M.M., and Markey, S.P.: Imipramine and desipramine in plasma and spinal fluid: relationship to clinical response and serotonin metabolism. Arch. Gen. Psychiat. 35: 621-625, 1978.

Major, L.F., Lerner, P., Goodwin, F.K., Ballenger, J.C., Brown, G.L., and Lovenberg, W.: DBH in cerebrospinal fluid: relationship to personality measures. Biol. Psychiat. 14: 337-345, 1979.

Post, R.M., Ballenger, J.C., and Goodwin, F.K.: Cerebrospinal fluid studies of neurotransmitter function in manic and depressive illness. In Wood, J.H. (Ed.): The Neurobiology of Cerebrospinal Fluid. New York, Plenum Press (in press).

Post, R.M., Gerner, R.H., Carman, J.S., Gillin, J.C., Jimerson, D.C., Goodwin, F.K., and Bunney, W.E., Jr.: Effects of a dopamine agonist piribedil in depressed patients: relationship of pretreatment HVA to antidepressant response. Arch. Gen. Psychiat. 35: 609-615, 1978.

Post, R.M., and Goodwin, F.K.: Approaches to brain amines in psychiatric patients: a reevaluation of cerebrospinal fluid studies. In Snyder, S., Iverson, S., and Iverson, L. (Eds.): Handbook of Psychopharmacology. New York, Plenum Press, 1978, pp. 147-185.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00448-06 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Urinary Norepinephrine Metabolites in Affective Disorders and Normal Controls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Rex Cowdry	Staff Psychiatrist	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinol.	CP/NIMH
	Thomas Wehr	Chief, Clinical Res. Unit	CP/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH
	Robert Post	Chief, Clinical Res. Unit	BPB/NIMH
	Dennis Murphy	Chief	CN/NIMH
	David Pickar	Staff Psychiatrist	CN/NIMH
	Giovanni Muscettola	Associate Professor	Univ. of Naples

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TOTAL MANYEARS: 1.12	PROFESSIONAL: 2.00	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This ongoing project evaluates a wide variety of factors which contribute to the urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), the norepinephrine metabolite thought best to reflect brain turnover. New findings or replications of earlier ones are as follows: (1) Despite an association between the circadian rhythms of MHPG and activity within depressed patients, total 24-hour activity does not correlate with 24-hour MHPG across patients; (2) In a large sample of patients and volunteers, depressed patients with a prior history of mania (bipolar I) have lower MHPG than normals, unipolar depressed or manic patients, although differences are modest; (3) There is a lack of correlation between anxiety and MHPG in our sample of depressed patients, nor is there a correlation between urinary and CSF MHPG; (4) Zimelidine, a serotonergic drug, is able to alter urinary MHPG to the same extent as does desipramine, a noradrenergic agent; (5) The dose response of desipramine-induced urinary MHPG decrease appears to be curvilinear instead of simply linear.

Project Description:

The purpose of this continuing project is an extensive study of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in patients and normal volunteers. MHPG is the metabolite thought best to reflect brain norepinephrine turnover. Recent clinical studies, by Ebert and Kopin in the LCS, using radioisotopes to study turnover indicate that a significant proportion of MHPG appearing in the urine originates from brain pools of norepinephrine.

We have continued a detailed investigation of factors that influence MHPG excretion including activity, diet, and anxiety. In order to use MHPG excretion as a tool to evaluate the hypothesis of altered norepinephrine function in depression and mania, the influence of such non-specific factors on MHPG excretion must be understood. State dependent changes in MHPG excretion may be of great importance as suggested by our earlier longitudinal studies in patients with rapid cycles.

The methods used for urinary MHPG studies in patients and normal volunteers have been previously described (see Z01 MH 00448-03). In brief, daily urine samples are collected either in 3-hour segments or as 24-hour pools, measured, refrigerated with preservative, aliquoted, frozen, and analyzed for MHPG. A 11 p.m.-7 a.m. urine collection substituting for a 24 hour sample is also under consideration. Areas of study may be divided into the following:

(1) Routine application of the patient activity monitor (PAM), a non-telemetric computer-based motor activity monitor, has continued. We have confirmed our earlier finding of an association between the circadian rhythm of MHPG and circadian level of activity within depressed patients. The total level of activity during a 24-hour period, however, does not correlate with urinary MHPG across patients.

(2) Using a reliatively large sample of depressed patients and normal volunteers it has been possible to replicate earlier findings of differences in 24-hour urinary MHPG between certain subtypes of depression, as well as to clarify relationships to a normal population. Findings are: in patients with bipolar illness, MHPG is higher in mania than in depression; bipolar I depressed patients have lower MHPG than either normal volunteers or depressed unipolar patients; and although statistically significant, absolute differences between groups are modest (15-20%).

(3) There has been a suggestion that levels of "anxiety" are associated with higher urinary MHPG. At least in depressed patients on placebo (N=68) we do not find this to be the case (r=0.06). This finding is important since it suggests that the differences between bipolar I depressed and unipolar depressed patients cannot be attributed to differences in anxiety. There is, however, a significant (p<.05) correlation between age and urinary MHPG (r=0.23) although this relationship explains only 5% of the variance. Since our volunteers tend to be younger, our data, uncorrected for age, may somewhat underestimate the absolute difference between normals and bipolar depressed. In keeping with the

dissociation of drug effects on urinary and CSF MHPG (see Z01 MH 01850-02 CP) there is no significant correlation between urinary and CSF MHPG in drug-free depressed patients (r=0.05; N=29). Finally, the severity of depression does not correlate with 24-hour urinary MHPG excretion, even in a large sample (N=66; r=-0.03).

(4) Drug-induced alteration in urinary MHPG has been a major focus of the drug cross-over studies. In one of the studies, a cross-over in the same patients between desipramine, a specific norepinephrine uptake inhibitor, and zimelidine, a specific serotonin uptake inhibitor, we were surprised to find that both drugs decreased urinary MHPG. If this finding is replicated, it will show that significant alterations in at least the metabolism (and perhaps turnover) of norepinephrine may be produced by drugs that have a primary action on the serotonin system. Another new finding was that desipramine has a different type of plasma drug concentration response curve for MHPG change in urine and CSF; in urine it appear to be curvilinear (i.e., at higher plasma concentrations changes are less) and in CSF simply linear (Z01 MH 01850-02 CP).

In collaboration with Drs. Murphy and Pickar (Clinical Neuropharmacology Branch) the effect of specific monoamine oxidase inhibitors (of type A and type B) on urinary MHPG has been investigated. Preliminary results reveal that at standard clinical doses, both types of drugs cause an 80% decrease in urinary MHPG. It may be, however, that at lower doses some differential effects may be demonstrated. This possibility is being currently investigated.

Goodwin, F.K. and Potter, W.Z.: Norepinephrine metabolite studies in affective illness. In Usdin, E., Kopin, I.J., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, Vol. II, pp. 1863-1865.

Goodwin, F.K. and Potter, W.Z.: Noradrenergic function in affective illness. In Saletu, B. (Ed.): Proceedings of the 11th CINP Congress. New York, Pergamon Press (in press).

Wehr, T.A., Muscettola, G., and Goodwin, F.K.: Urinary MHPG circadian rhythm: early timing (phase advance) in manic-depressives compared with normal subjects. Arch. Gen. Psychiat. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01835-02 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral and Biochemical Effects of Endorphin Agonists and Antagonists in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Steven Paul	Clinical Associate	CP/NIMH
	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
	Alfred Lewy	Staff Psychiatrist	CP/NIMH
	Ron Schoenfeld	Guest Scientist	CP/NIMH
	William J. Bunney, Jr.	Chief	BPB/NIMH
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COOPERATING UNITS (if any)
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TOTAL MANYEARS: .70	PROFESSIONAL: 1.25	OTHER:

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are interested in the role of endogenous opiate peptides, called endorphins, in central nervous system functioning in general and in mental illness in particular. Much of our work has centered around FK 33-824, a synthetic enkephalin analogue which can be administered parenterally and orally and hence may be of use clinically. Animal studies have included demonstration of FK 33-824 inhibition of conditioned avoidance responding in rats and blockade of anxiety behavior and increase in serum prolactin in monkeys. These results suggest an interaction of FK 33-824 with brain dopamine and norepinephrine systems and possible antipsychotic and antianxiety properties in man. Clinical trials of FK 33-824 are planned. A controlled trial of the morphine like compound methadone in affective illness and schizophrenia is in its early stages. Also, the Branch is participating in collaborative double-blind controlled trials of beta-endorphin and of the opiate antagonist naloxone. We find little or no effect of naloxone in mania.

Project Description:

Endorphins are endogenous opiate peptides found in the brain. There is a growing body of laboratory and clinical evidence that endorphins play a role in important CNS functions, including pain perception, mood, behavior, and neuroendocrine functioning. Much of our work has involved the use of FK 33-824, an analogue of the naturally occurring pentapeptide methanine enkephalin, synthesized by Sandoz, Ltd. Because FK 33-824, in contrast to the natural endorphins, can be synthesized and administered with relative ease, it is a drug which might be of use clinically. It has been administered safely to volunteers in Europe.

We have been investigating the pharmacology of the endorphin system in a number of ways, including behavioral and biochemical studies of endorphin agonists in animals and clinical and neuroendocrine studies of endorphin agonists and antagonists in humans. Studies of the behavioral effects of FK 33-824 in animals include: 1) comparison of FK 33-824 induced catatonia in rats to that produced by morphine and haloperidol; 2) observation in rats of behavioral effects of FK 33-824, including its effects on dopamine mediated stereotyped behavior induced by apomorphine and amphetamine; 3) inhibition by FK 33-824 of conditioned avoidance responding in rats; 4) relationship between adrenal steroids, pituitary ACTH and endorphin release and the behavioral effects of FK 33-824 and apomorphine; 5) blockade by FK 33-824 in monkeys of piperoxan-induced anxiety behavior, which is mediated via activation of the noradrenergic locus coeruleus in the brain (in collaboration with Drs. Gold and Redmond at Yale). Biochemical studies of FK 33-824 include: 1) measurement of amine metabolites in monkey CSF; 2) demonstration of an increase in serum prolactin in monkeys after FK 33-824. The inhibition of conditioned avoidance and elevation of prolactin are consistent with an antagonistic effect of FK 33-824 on dopamine systems and suggest possible antipsychotic efficacy for the compound.

In rats intraperitoneal injection of FK 33-824 produces apomorphine-like stereotyped behavior. FK 33-824 induced stereotypy is antagonized by naloxone and neuroleptics, indicating that this drug can activate both opiate and dopamine receptors in the brain. Because increased dopaminergic neuronal activity is thought to be involved in schizophrenia and dopamine mediated stereotypy has been used as an animal model for this illness, these results are consistent with an involvement of endogenous opiate-like peptides in schizophrenia. These findings may provide a possible mechanism for the reported improvement in schizophrenic psychosis produced by naloxone.

There is a preliminary report of improvement in a variety of major psychiatric disorders after administration of beta-endorphin. Following up on this, we are collaborating with the Biological Psychiatry Branch of the NIMH in a double-blind placebo controlled study of the effects of beta-endorphin administered intravenously to patients with affective disorder and schizophrenia. The beta-endorphin is being supplied by Dr. Li. In addition to intensive observation of mental status, the effects on pain sensitivity and neuroendocrine parameters are being measured. A similar clinical trial of FK 33-824 is planned pending receipt of an IND from the FDA.

We have administered the specific opiate antagonist naloxone in humans. Presumably, changes produced by naloxone administration involve the endorphin system. In order to elucidate the role of endorphins in neuroendocrine regulation, naloxone (20 mg) has been injected s.c. in male volunteers and serial blood samples taken for assay of a variety of hypothalamic hormones. A similar study in female volunteers is planned, as is a study of naloxone effects on a TRH infusion.

Following up on a report that naloxone caused improvement in a subset of manic patients, we collaborated with BPB in administering naloxone 20 mg s.c. to ten manic patients with a placebo controlled, double-blind design. In only one of the ten patients was any positive effect observed.

A controlled trial of methadone, a synthetic morphine-like drug, has been undertaken in patients with affective illness and schizophrenia. Both intravenous and oral routes of administration are being evaluated. So far only a few patients have received the drug, but preliminary results are encouraging.

Publications:

Extein, I., Goodwin, F.K., Lewy, A.J., Schoenfeld, R.I., Fakhuri, L.R., Gold, M.S., and Redmond, D.E., Jr.: Behavioral and biochemical effects of FK 33-824, a parenterally and orally active enkephalin analogue. In Usdin, E. (Ed.): Endorphins in Mental Health Research. London, McMillan, 1979, pp. 279-292.

Extein, I., Tallman, J. and Goodwin, F.K.: Changes in lymphocyte beta-adrenergic receptors in depression and mania. Psychiatry Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01850-02 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Clinical Pharmacology of Antidepressants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Sanford Markey	Chief, Pharm. Appl. of Mass Spec.	LCS/NIMH
	Irwin J. Kopin	Chief	LCS/NIMH
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	Judith Rapoport	Staff Psychiatrist	BPB/NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.40	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although tricyclic antidepressants are very widely used in clinical practice the clinical pharmacology of these compounds is not well-understood. The purpose of the studies described in this report is to provide guidelines for the rational use of these drugs in the treatment of depression. Major foci during the past year include (1) pharmacokinetic prediction of steady-state concentrations; (2) assessing the relevance of factors such as age, sex and RBC/plasma ratios to steady-state pharmacokinetics; (3) identifying active hydroxylated metabolites in adults and children; (4) comparing biochemical and clinical effects of a specific norepinephrine uptake inhibitor (desipramine) and a specific serotonin uptake inhibitor (zimelidine); (5) describing the pharmacokinetics of zimelidine in depressed patients; (6) investigating the cardiovascular, biochemical and behavioral effects of desipramine in normal volunteers; and (7) developing an animal model of testing the chronic effects of antidepressants on beta receptors by measuring plasma melatonin.

Project Description:

Research focusing on the clinical pharmacology of antidepressants has become possible following the development of a sensitive GC-MS assay in collaboration with Dr. Markey of the Laboratory of Clinical Science. Clinical application of this assay was initiated by Dr. Muscettola (a former Visiting Scientist) in this laboratory in collaboration with Drs. Zavadil and Markey - a high correlation was demonstrated between plasma and CSF concentrations of imipramine and desipramine (see July '76 - Sept. '77 Report Z01 MH 00446-08 CP).

Given the means of reliably measuring drug concentration, many studies have been undertaken. Rapid progress has continued with the participation of Dr. Calil, a W.H.O. Fellow who joined the laboratory in October, 1977. The viability of the program has been recognized by designation of Dr. Goodwin as a preceptor for the NIGMS-sponsored clinical pharmacology program (PRAT program). An M.D.-Ph.D. psychiatrist matched with our Branch for a NIGMS-PRAT Fellowship, and has joined our Branch as of July, 1979. A Ph.D. neuropharmacologist has also matched and received a PRAT Fellowship to begin August 16, 1979. And an additional M.D.-Ph.D. psychiatrist has received a PRAT Fellowship to begin July 1, 1980.

Two distinct approaches have been defined: I) Pharmacokinetic analysis designed to provide maximally effective utilization of proven antidepressants and II) comparisons of clinical and biochemical effects of specific antidepressants in patients and normals as a means of studying the biochemistry of major affective disorders.

The major pharmacokinetic studies are as follows:

(1) On the basis of our success using single oral doses to predict ultimate steady-state concentrations (Z01 MH 01850-01 CP) we have applied this test in an almost routine manner to the tricyclic antidepressant desipramine. Because the secondary amine, desipramine, has somewhat "simpler" pharmacokinetics than the tertiary amine, imipramine, both "single-point" and area under the curve predictions yield accurate predictions of steady-state.

(2) Since our inpatients are free of other illnesses and live in a controlled environment we have a unique opportunity to study the steady-state pharmacokinetics of tricyclics. A number of important findings have emerged: inter-individual variation in plasma concentration for a given dose is not as great as previously reported; an age effect on the ability to metabolize tricyclics is not apparent in our population of patients (ages 18-67); under controlled conditions an individual's ability to metabolize tricyclics is a stable "trait"; reported variations in RBC/plasma ratios of tricyclics are, in part, an "artifact" resulting from differential equilibration between these two tissues during dosing intervals.

(3) A collaboration with the Unit on Childhood Illnesses, BPB (Dr. Rappaport) has permitted a demonstration that pre-pubescent boys more actively

demethylate the tertiary amine tricyclic, imipramine, than adults. Overall, however, the total metabolism is not much different from adults. If anything, children achieve lower ultimate steady-state concentrations of tricyclics than do adults for a given mg/kg dose.

(4) Method development has continued with our laboratory providing informal consultation to commercial laboratories in the area so that they can improve the quality of their measurements. Following validation on the CG-MS, we now feel confident of the HPLC method developed by Dr. Jusko (Z01 MH 01850-01 CP) and are establishing this method in our own laboratory in order to do the more routine analyses.

(5) By measuring tricyclic concentration in the CSF (that is not protein bound) we have demonstrated that total steady-state plasma concentration provides a reasonable estimate of free drug. In our recent studies with desipramine, a 0.94 correlation between total plasma and CSF concentrations was found.

(6) In initial investigations of the non-tricyclic antidepressant, zimelidine, a serotonin uptake inhibitor, we have found that there is a much closer correspondence between dose and plasma concentrations of the major active form of the drug (norzimelidine) than is the case with tricyclic antidepressants. The plasma half-life and thus, the time necessary to reach steady-state concentrations of norzimelidine is very similar to that observed with the major tricyclics. Zimelidine has a relatively short half-life and has such wide intradose variations using a standard dosing regimen that reasonable steady-state measurements are only possible using intradose area under the curve measurements. These findings are of obvious consequence for planning studies that attempt to relate the concentrations of zimelidine and norzimelidine to therapeutic or biochemical effects (see below).

With respect to comparisons of clinical and biochemical effects of antidepressants, recently completed and current studies include the following:

(a) Eight patients have completed a unique double-blind cross-over trial in which an experimental antidepressant (zimelidine), specific for serotonin uptake inhibition, is compared to an established antidepressant (desipramine) specific for norepinephrine uptake inhibition. Initial findings include a failure of zimelidine to precipitate mania or rapid cycles in patients (as contrasted to desipramine); no overall difference in the antidepressant efficacy of the two drugs despite differential effects in MHPG and 5HIAA in the CSF (see Z01 MH 00447-10 CP); an unexpected lowering of urinary MHPG following zimelidine almost as great as that seen after desipramine (see also Z01 MH 00448-06 CP) and a positive correlation between concentration of norzimelidine (the truly specific serotonin uptake inhibitor) and changes in self-rating scores, Hamilton Ratings and Bunney-Hamburg Ratings. Additional studies are underway in an attempt to replicate these findings.

(b) A closer investigation of the relationship between steady-state concentration of desipramine and MHPG change in the urine and CSF has revealed an interesting dissociation. In the CSF the extent of decrease of MHPG is positively correlated with desipramine, whereas in the urine, except at low desipramine concentrations, the MHPG decrease shows a weak negative correlation with drug level.

(c) The recent suggestion from this laboratory that hydroxylated metabolites of tricyclics may be active has received considerable support from continued studies as well as evidence presented by colleagues at the Karolinska Institutes that hydroxynortriptyline is the predominant from form found in man following nortriptyline. We have now shown that although hydroxy-desipramine is not on the average the predominant form in adults or children, it can be a major proportion in some individuals and is present in the CSF to at least an equal extent relative to plasma concentration as desipramine. Moreover, accounting for hydroxy-desipramine improves the correlation between drug concentration and antieneuretic effect in children and extent of MHPG decrease in CSF of adults.

(d) The assessment of the acute and chronic effects of "therapeutic" concentrations of desipramine in normal volunteers is underway. In collaboration with Dr. Kopin, the effects of desipramine on plasma norepinephrine and all of its significant metabolites will be measured both in terms of the subject at rest and of the change seen on going from a supine to standing position. These measurements may yield the information necessary to understand some of the cardiovascular effects of tricyclic antidepressants. In addition, the effects of desipramine on beta-adrenergic function as reflected in plasma melatonin (Z01 MH 01851-02) and on hypertonic saline-induced vasopressin release (Z01 MH 00452-04) are being assessed.

(e) The results of acute and chronic zimelidine and desipramine on multiple hormones has also been evaluated. In contrast to some previous observations, acute desipramine has no significant effect on growth hormone, leutinizing hormone, prolactin and urinary free cortisol. Moreover, the serotonergic zimelidine also does not influence any of these parameters. Implications of these findings are being explored by the studies as described on Z01 MH 00452-04.

Finally as part of the clinical experiments designed to understand the pharmacology of antidepressants, a number of chronic studies in rats have been undertaken. These focus on a study of the relationship between beta-receptor alterations in brain and pineal gland, drug concentration and plasma melatonin. Preliminary findings indicate that beta-receptor preparations from animals given desipramine contain very high concentrations (approximately 1.0 micromolar) of drug. This is of interest since it has been assumed that the presence of desipramine does not relate to decreased beta receptors; it now appears appropriate to reassess this assumption. Moreover, in an initial experiment, the degree of change of melatonin secretion (either spontaneous or isoproteronol-induced) does not appear to correlate well with desipramine-induced beta-receptor changes.

Publications:

Potter, W.Z., Zavadil, A.P., and Goodwin, F.K.: Prediction of steady-state plasma concentration of imipramine. Psychopharmacol. Bull. 14: 29-33, 1978.

Potter, W.Z., Muscettola, G., and Goodwin, F.K.: Binding of imipramine to plasma protein and to brain tissue: relationship to CSF tricyclic levels in man. Psychopharmacol. 63: 187-192, 1979.

Potter, W.Z., Calil, H.M., Manian, A.A., Zavadil, A.P., and Goodwin, F.K.: Hydroxylated metabolites of tricyclic antidepressants: preclinical assessment of activity. Biol. Psychiat. 14(4): 601-613, 1979.

Potter, W.Z., Calil, H.M., Mannian, A., and Goodwin, F.K.: Hydroxylated metabolites of tricyclic antidepressants: inhibition of amine uptake and other preclinical studies. In Usdin, E., Kopin, I., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 373-375.

Potter, W.Z., Calil, H.M., Zavadil, A.P., Jusko, W.J., Sutfin, T., Rapoport, J.L., and Goodwin, F.K.: Steady-state concentration of hydroxylated metabolites of tricyclic antidepressants in patients: relationship to clinical effect. Psychopharmacol. Bull. (in press).

Rapoport, J.L. and Potter, W.Z.: Tricyclic antidepressants and children. In Raskin, A. (Ed.): The Difference of Age on the Pharmacology of Psychoactive Drugs. New York, Raven Press (in press).

Potter, W.Z., Zavadil, A.P., Kopin, I.J., and Goodwin, F.K.: Single dose kinetics predict steady-state concentrations of imipramine and desipramine in patients. Arch. Gen. Psychiat. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00452-04 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Studies of Major Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Emanuel deFraites	Clinical Associate	BPB/NIMH
	Seymour Reichlin	Chief, Division of Endocrinol.	Tufts Univ.
	Detlev Ganten	Prof., Dept. of Pharmacol.	U. of Heidelberg
	Gregory Brown	Chief, Dept. of Neurosciences	McMaster Univ.
	Gary Brown	Professor of Medicine	Univ. of Chicago
	Samuel Yen	Chief, Dept. of Reproductive Endocrinology	UCSD
	Walter Kaye	Clinical Associate	LCS/NIMH
	Michael Ebert	Chief, Section on Exp. Ther.	LCS/NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.40	2.50	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hormones of the hypothalamic-pituitary axis (HP) exert direct behavioral effects, by activation of specific receptors in brain or by modulation of central neurotransmitter systems. The synthesis and release of HP hormones is partially under the control of the monoaminergic neurotransmitters and is psychoactive agents. Current studies concentrate on measurement and administration of behaviorally active peptides, particularly vasopressin (VP), as well as on comprehensive tests of HP function, including dopamine infusion. Major findings this year are: CSF VP is significantly higher in drug free manic compared with drug free bipolar depressed patients, while it tends to be lower in bipolar depressed patients compared with controls. The threshold and sensitivity of the VP response to sustained hypertonic saline infusion is greater in bipolar patients during the manic phase than the depressed phase. DDAVP, a specific analog of VP, produced significant amelioration of depression and psychosis in some patients with affective illness; in addition, DDAVP significantly enhanced the formation, encoding, and organization of long-term trace events in memory in both patients and control subjects.

Project Description:

Several aspects of the symptom complex of the functional psychoses, particularly affective illness, suggest hypothalamic dysfunction. Thus, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such as amenorrhea. Interest in the hypothalamic-pituitary (HP) axis has been further stimulated by recent findings that the monoamine neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, these peptides and hormones may have specific receptors in brain and have been shown to affect the brain neurotransmitter systems. Several of these hormones have also been shown to be responsible for specific behavioral effects.

Several neuroendocrine strategies are currently in use: (1) direct measurement in CSF and in plasma of behaviorally active peptides following stimulation according to verified stimulation paradigms; (2) dopamine infusions and administration of various hypothalamic releasing factors to test responses of the HP axis, and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction in patients with affective illness, schizophrenia, and anorexia nervosa; (3) psychoactive drug effects on HP function and on the levels of behaviorally active peptides; (4) assessment of the temporal organization of neuroendocrine function; and (5) assessment of the relationship between neuroendocrine function and sleep.

Because of limited laboratory support personnel and the large volume of assays required to complete this neuroendocrine project, the Unit on Neuroendocrinology maintains collaborative and contractural arrangement with several university research facilities for neuroendocrine assays. We are fortunate to collaborate with scientists of outstanding caliber, including Dr. Seymour Reichlin, Dr. Samuel Yen, Dr. Gregory Brown, Dr. Gary Robertson, and Dr. Detlev Ganten.

1. Studies of behaviorally active CNS peptides:

a. The role of the CNS peptide vasopressin (VP) is currently under study, particularly in patients with affective illness. In a major published review, we formulated an hypothesis suggesting a role for vasopressin in the regulation of affect in man. The review stressed the functional relationship between vasopressin and many aspects of the symptom complex of affective illness, including alterations in the consolidation of memory, modulation of pain sensitivity, synchronization of biological rhythms, timing and quality of REM sleep, and the regulation of fluid and electrolyte balance. We concluded that the majority of the available data in experimental animals suggested, as a first approximation, that central vasopressin function is relatively augmented in mania compared with depression.

Our first quantitative studies of vasopressin function in man involved measurement of CSF vasopressin levels in both patients and control subjects. The CSF seems to be a major communication pathway for conveying VP to various areas of the brain where it may modulate cell aggregates controlling one or more physiological process. Preliminary findings in untreated manic-depressive subjects show that CSF VP is significantly higher in manic patients than in depressed patients or controls, while there is a trend towards lower values in bipolar depressed patients compared with normals.

An osmoreceptor located within the hypothalamus is mainly responsible for the regulation of plasma vasopressin levels. The centrally mediated set point to osmostic stimuli reflects the cumulative effects of various inputs, either peptidergic (e.g., endorphinergic) or monoaminergic that regulate osmoreceptor function. We have evaluated osmoreceptor function in patients and controls by measuring the VP response to a sustained carefully timed I.V. infusion of hypertonic saline. It has been shown that a linear relationship exists between VP levels and increasing plasma osmolality if hypertonic saline is infused at the rate of .05 ml/kg/min. We have demonstrated that the correlation between VP and osmolality (sensitivity) and the threshold for VP secretion is reproducible within a given individual and is thus, a reliable biologic marker when followed longitudinally. It has also been demonstrated that the threshold and sensitivity for VP secretion seem to be heritable, since as they are virtually identical in identical homozygous twins. In studies of patients with affective illness patients in the manic state show greater sensitivity of VP responses to hypertonic saline than when they are depressed. This finding, in association with elevated CSF VP in mania compared with depression, is compatible with our initial hypothesis of relatively augmented VP function in mania compared with depression.

Lithium, a vasopressin receptor blocker, significantly augments the VP response to hypertonic saline. On the other hand, carbamezapine, a VP receptor agonist significantly reduces the VP response to hypertonic saline. Each of these drugs has been reported to have both antimanic and antidepressant effects and to exert an overall prophylactic effect on affective episodes. Both lithium and carbamezapine appear to initiate compensatory changes in vasopressin secretion in the direction opposite to their effects on the vasopressin receptor; possibly these drugs function by circumscribing the range of central vasopressin action in a manner analogous to their effects on other regulated systems. In this formulation drug efficacy is related to stabilization of the functional activity of a central peptidergic system.

To study the possible relationship of VP to affective illness, we have initiated a double-blind placebo controlled trial of the VP analog DDAVP in patients with major affective illness. Preliminary data show that DDAVP induces highly significant and consistent improvements in measures designed to quantify the formation, encoding, and organization of long-term trace events in memory. Some patients also showed significant amelioration of depressive and psychotic symptomatology during DDAVP treatment. The effect of DDAVP and other VP analogs is also being studied in patients with dementia and schizophrenia, and in control subjects.

In collaboration with Dr. Walter Kaye and Dr. Michael Ebert of the LCS we have evaluated VP function in patients with anorexia nervosa. These patients show a syndrome of partial diabetes insipidus, although this finding was previously demonstrated only by indirect means. The majority of patients at 70% ideal weight showed markedly diminished sensitivity of VP responses and a return to normal secretory capacity when weight returned to normal. These patients show a reversal of the normal CSF/plasma VP ratio, which is also seen in some patients with idiopathic diabetes insipidus. The cause of this apparent hypersecretion of the central VP pathway is unknown, but is of interest in view of the possible behavioral effects of VP in man.

In collaboration with Dr. Robert Post and Dr. James Ballenger of the BPB, we have studied CSF VP in control subjects. This study was undertaken to provide normative data for comparison with our patient populations. Each subject also received a battery of cognitive and psychological tests shortly following lumbar puncture. A significant positive correlation between CSF VP and tests of memory function was noted.

b. CSF levels of angiotensin I, angiotensin II, and angiotensinogen are being studied in collaboration with Dr. Detlev Ganten. Angiotensin is a central peptide that modulates fluid and electrolyte homeostasis. It influences learning processes in experimental animals, and is involved in perception of pain and pleasure. Levels of the enzyme that catalyzes the conversion of angiotensin I to angiotensin II have been reported to be deficient in the brains of schizophrenic subjects studied post-mortem. Preliminary data in our schizophrenic subjects is compatible with this finding; we show elevated levels of CSF angiotensin I in these patients compared with normals.

c. CSF somatostatin levels are studied in collaboration with Dr. Seymour Reichlin. We find CSF somatostatin levels are higher in manic patients than in bipolar depressed patients.

d. Neuroendocrine effects of opiate agonists and antagonists: Evidence from experimental animals suggests that the endorphinergic system plays an important role in the modulation of a variety of neuroendocrine systems, including vasopressin, prolactin, growth hormone, the gonadotropins, and cortisol. However, in patients large doses of naloxone do not affect the VP response to hypertonic saline, baseline LH, FSH, prolactin, and growth hormone secretion, or the prolactin and TSH response to TRH infusion. Acute methadone and β-endorphin infusion in depressed patients causes marked prolactin stimulation and moderate growth hormone release. The cortisol suppression following methadone infusion is of interest in light of the common peptide precursor for both ACTH and β-endorphin and the suggestion of a common feedback loop subserving secretion of these two hormones.

2. Dynamic tests of monoaminergically regulated HP systems:

a. A major emphasis this year is the evaluation of responses to a three hour infusion of dopamine (DA) of patients with affective illness, schizophrenia, anorexia nervosa, and control subjects. In collaboration with Dr. Gregory Brown, we have shown in normals a significant LH decrement during DA infusion followed by a rebound at the end of the infusion; the LH response reflects the functional activity of hypothalamic DA receptors, while the prolactin response reflects the status of both hypothalamic and peripheral (pituitary) DA receptors.

Peak plasma DA levels are achieved after one and one-half hours, after which they fall substantially despite continued DA infusion. A possible explanation for the fall in DA levels may be the significant elevations in both platelet and plasma MAO during the DA infusions. Drug free psychotic schizophrenic patients show significantly greater LH suppression during DA infusion with absent LH rebound following the infusion, while drug free manics show a paradoxical LH rise during DA infusion. The data on schizophrenic patients suggest hypersensitivity of some hypothalamic DA receptors; the paradoxical LH response in manic patients suggests an abnormality of DA mediated regulatory mechanisms. Prolactin responses to DA infusion do not differ among schizophrenics, affective patients and controls.

In schizophrenic patients, LH responses are elevated following withdrawal of pimozide (a DA receptor blocker) in contrast to prolactin responses that diminished during the pimozide withdrawal period. These data are compatible with those in experimental animals that show that during a neuroleptic withdrawal period hypothalamic DA receptors become supersensitive while pituitary receptors demonstrate subsensitive responses.

In collaboration with Dr. Walter Kaye and Dr. Michael Ebert of the LCS, we have also monitored LH and PRL responses to DA infusion in patients with anorexia nervosa. At 70% ideal weight, anorexics show a blunted LH response to DA infusion, suggesting an already maximally (dopaminergically) suppressed LH status in these patients. These data are in agreement with previous hypotheses linking hypothalamic DA excess to anorexia nervosa.

b. Neuroendocrine effects of apomorphine are being studied in normal subjects and in patients with affective illness and schizophrenia. Apomorphine, a peripheral and central dopamine receptor agonist induces an LH decrement in controls and psychiatric patients, analogous to that produced by a sustained DA infusion.

c. We are evaluating our results from an integrated neuroendocrine procedure that uses arginine, a central releaser of growth hormone and prolactin, the hypothalamic peptide TRH, a releaser of prolactin and thyrotropin stimulating hormone, and LH-RH that releases LH and FSH. The purpose of the timed infusion of these three trophic substances is to provide an evaluation of overall HP function. Data from a large number of patients in various clinical states and treated with various medications are now being analyzed. Plasma levels of the adrenal steroid, dihydroepiandrosterone sulphate were found to be decreased in bipolar depressed women compared with control subjects and to be disproportionally low compared with a simultaneously drawn plasma cortisol level.

d. Tests of the hypothalamic-pituitary-adrenal axis in affective illness and schizophrenia have focused on urinary free cortisol (UFC) excretion with and without dexamethasone suppression. Psychotic unipolar patients have a very high UFC when compared with non-psychotic unipolar patients or with bipolar depressed patients. Bipolar depressed patients show a significantly higher UFC than bipolar patients in the manic phase. Among unipolar patients, UFC showed a significant positive correlation with CSF 5HIAA accumulation after probenecid. This finding is compatible with data in experimental animals that suggests that serotonin is excitatory to HPA function. Thus we initiated a trial of the serotonin receptor blocker cyproheptadine in depressed patients with hypercortisolemia. The first depressed patient to receive cyproheptadine had an antidepressant response and a marked drop in cortisol excretion. Within a few days, however, the patient began rapidly cycling between hypomania and depression. The hypomanias were associated with low while the depressions with high cortisol production. The reason for this cyproheptadine induced rapid cycling remains unclear. Further trials of cyproheptadine in depressed patients with affective illness and HPA activation are now underway.

A blunted growth hormone response to amphetamine administration was reported in patients with endogenous depression. It has been suggested that the mechanism for this blunted response is due to diminished catecholaminergic function. In collaboration with Dr. Elliot Gershon and Dr. John Nurnberger of the BPB, we are studying the reproducibility and heritability of neuroendocrine responses to amphetamine in a population of normal homozygous and heterozygous twins. Preliminary data shown that the GH and prolactin responses are heritable and reproducible.

3. Assessment of psychoactive drug effects on HP function: The effect of commonly used psychoactive agents such as lithium, imipramine, and MAO inhibitors, as well as experimental drugs such as carbamezapine piribedil, pimozide, zymelidine, and methadone are being assessed on responses to each of our neuroendocrine challenge paradigms, including DA infusion, the integrated infusion (arginine, TRH, LH-RH), apomorphine stimulation, and hypertonic saline infusion. Most of these studies are nearing completion. Some preliminary findings show that chronic treatment with the dopamine agonist, piribedil, reduces growth hormone responses to arginine and apomorphine, suggesting a down regulation of central dopaminergic receptors. Both chronic desmethylimipramine and zymelidine treatment fail to affect basal LH, FSH, prolactin, cortisol, or growth hormone secretion. These results are of practical significance because they show that chronic treatment with tricyclic antidepressants does not disrupt neuroendocrine homeostasis; theoretically, it is of interest that these drugs fail to significantly alter neuroendocrine secretion even though they significantly alter turnover of biogenic amines that modulate these neuroendocrine systems. These findings suggest that chronic tricyclic treatment, although altering biogenic amine turnover, induces compensatory changes in amine function that promote homeostasis in the monoaminergic systems, reflected in maintenance of homeostasis in neuroendocrine processes.

We have also examined the acute neuroendocrine effects of a series of ECT treatments in depressed patients. Preliminary findings show that ECT induces rapid release of vasopressin, prolactin, and cortisol, but not growth hormone. The cortisol response becomes progressively less pronounced after ECT treatment once some clinical improvement has occurred.

4. Relationship between neuroendocrine function and sleep: Neuroendocrine function is characterized by distinct diurnal and nyctohemeral variations; the nocturnal variations of some hormones, notably growth hormone are related to the specific stage of sleep. Conversely, the timing of certain sleep stages may be related to the release of hypothalamic or pituitary hormones. In collaboration with W. Mendelson and C. Gillin we have found that growth hormone administration significantly reduces slow wave sleep and enhances REM sleep.

5. Evaluation of gonadal endocrine dysfunction in patients with affective illness: "Psychogenic" amenorrhea is a frequent but poorly understood phenomenon which frequently occurs in patients with affective illness, particularly during the depressed phase. Depressed amenorrheic women show significantly higher CSF 5HIAA compared with those with regular menstrual cycles, and these amenorrheic women also show a blunted TSH response to TRH. Taken together, these data suggest that depressed amenorrheic women have increased functional serotonergic activity, in agreement with animal evidence that serotonin exerts an inhibitory role on gonadotropin release.

We have noted the occasional appearance of 'paradoxical' hyperthyroidism in patients receiving lithium therapy in the outpatient clinic. The hyperthyroidism may be subclinical, detectable only by repeated thyroid function studies. It is of interest that these episodes of subtle hyperthyroidism are often accompanied by a manic episode. The mechanism of this hyperthyroidism and the associated breakthrough of mania are under study.

Publications:

Gold, P.W., Ballenger, J.C., Zis, A.P., Post, R.M., and Goodwin, F.K.: A vasopressin hypothesis of affective illness: Preliminary findings. In Usdin, E., Kopin, I.J., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, Vol. II, pp. 1176-1178.

Gold, P.W. and Robertson, G.L.: Central peptide regulation of fluid and electrolyte balance: Psychiatric implications. In Alexander, P. (Ed.): Fluid and Electrolyte Disturbances in Psychiatric Illness. New York, Spectrum Press, in press.

Reus, V.I., Gold, P.W., and Post, R.M.: Lithium-induced thyrotoxicosis. Am. J. Psychiatry 136: 724-725, 1979.

Gold, P.W., Extein, I., Ballenger, J.C., and Wehr, T.: Rapid mood cycling and concomitant cortisol changes induced by a serotonin antagonist. Am. J. Psychiatry, in press.

Gold, P.W.: Contemporary endocrine-behavioral interactions. In Flach, S. (Ed.): Current Issues in Psychobiology, in press.

Gold, P.W., DeFraites, E., Zis, A.P., van Kammen, D., and Goodwin, F.K.: The LH response to dopamine infusion: Possible marker for central dopaminergic function in psychiatric patients. Psychopharmacol. Bull. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00449-05 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Outpatient Followup Studies of Manic-Depressive Patients and Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
OTHER:	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
	Frederick Goodwin	Chief	CP/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Gay Grover	Clinical Nurse Expert	CC Nursing
	Paul Grof	Professor of Psychiatry	McMaster U.
	Leon Cytryn	Medical Officer (Research)	BP/NIMH
	Martine Lamour	Guest Worker	BP/NIMH
	Marion Yarrow	Chief	LDP/NIMH
	Carolyn Waxler	Research Psychologist	LCP/NIMH
	Elliot Gershon	Chief, Unit on Psychogenetics	BP/NIMH
	Eleanor Dibble	Social Worker	BP/NIMH

COOPERATING UNITS (if any)
Unit on Psychogenetics, BP/NIMH; Clinical Center Nursing; Extramural Clinical Research Branch, NIMH; McMaster University, Hamilton Ontario; Parent Child, Inc., Wash., D.C.; UCLA, Los Angeles, California; LDP/NIMH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient facility is a treatment and research resource for a variety of studies related to manic-depressive illness such as: (a) biological studies of the "well-state"; (b) the use of monoamine oxidase inhibitors to treat depression in bipolar patients on lithium; (c) investigation of the attitudes toward and perceptions of manic-depressive illness in bipolar patients compared with their spouses; (d) exploration of the efficacy of couples group therapy as an adjunct to lithium maintenance; (e) the study of pregnancy and the post-partum period in bipolar patients and their spouses; (f) the study of child development and affective response of infants with a bipolar parent; (g) the study of families with multigenerational manic depressive illness; (h) a retrospective analysis of longitudinal data on 334 patients, focusing on the natural course of the illness, with particular emphasis on unipolar-bipolar differences.

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Morris Parloff	Chief, Psychotherapy & Behavioral Interventional Center	CR/NIMH
Mariana Webster	Nurse Specialist	Parent Child
Gabrielle Carlson	Acting Director, Child Psychiatry Training Program	Neuropsych. Inst., UCLA

Project Description:

A reorganization of the Outpatient Clinic is in progress under the direction of its new Chief, Dr. Rex Cowdry. Followup studies now involve 60-70 outpatients who are seen in a variety of treatment modalities: a weekly and a monthly couples' group, a weekly psychotherapy group, three monthly lithium maintenance groups, and a series of special project groups (such as the study of pregnancy in bipolar patients and the subsequent study of their infants). Limited individual therapy is available. Crisis intervention approaches are used.

The availability of long-term follow-up data for large numbers of patients with affective disorder is of critical importance to our understanding of the basic illness process and therefore, to the interpretation of the increasing number of biochemical, genetic and pharmacotherapeutic findings. For the clinician, the choice of whether or not to place a given patient on prophylactic lithium maintenance involves at least an implicit estimate of what the natural course of the illness would be in the absence of the drug. Knowledge of the natural course of the illness is essential if he is to make appropriate decisions concerning long-term prophylaxis. It is known that lithium maintenance is not always effective in preventing recurrences of bipolar illness, a finding due undoubtedly to a variety of illness related factors, as well as psychosocial issues (stresses and/or non-compliance).

As one approach to these questions we have been studying the impact of adjunctive couples group psychotherapy on the prophylactic efficacy of lithium in bipolar patients. To assist us in these efforts we are collaborating with Morris Parloff and his staff in the Extramural Clinical Research Branch; our assessment techniques are compared with those to be used in the extramural studies of drug-psychotherapy interactions.

The couples' groups also provide a major and rich resource for the study of the relationship of the illness in individual family members to overall family pathology. The weekly couples' group focuses on couples in whom the husband has "dysporsic" manias, providing an opportunity to explore the phenomenology of that particular presentation of the manic syndrome, and the distinctive dynamics that may develop both between the patient and spouse and within the couples' group. The monthly couples' group provides a focus for the study of multigenerational bipolar illness. An article on the psychodynamic features of these families was published this year in the Amer. J. Orthopsychiatry.

A collaborative study with the Psychogenetics Unit of the BPB has been completed; in this study attitudes and perceptions of manic-depressive illness in families were examined, using patients in our couples groups. Our findings (Targum et. al.) were that 53% of well spouses compared to 5% of bipolar index patients indicated they would not have married, and 47% of well spouses compared to 5% of the patient group would not have had children had they known more about the illness prior to marriage. The overall data suggest that the patients, when compared with their spouses, minimize the burden of illness, and deny the heritable/familial nature of affective illness. These findings have implications for genetic counselling, as well as for psychotherapeutic treatment.

Another group followed in the clinic this past year as part of a joint collaborative study with the Psychogenetics Unit consisted of six bipolar couples who were expectant parents. Three of the female index patients were withdrawn from lithium carbonate maintenance in the clinic when they indicated a wish to become pregnant. In the fourth month of the pregnancies, the clinic sponsored a childbirth education group, with the participation of an RN childbirth educator from Parent Child, Inc. Evening sessions were held in which patients and their spouses participated. The couples completed a course in prepared childbirth, and discussed the psychology of pregnancy, the syndrome of post-partum "blues", and the high risk for the occurrence of post-partum affective disorder in this group of patients. Although it was our original hope to maintain the highly vulnerable patients off lithium through lactation, two of them developed post-partum manic episodes resulting in the hospitalization of one. A manuscript is in press describing this experience, the various psychodynamic features and previous treatment history of the three patients, and offering recommendations for the care and treatment of bipolar patients considering parenthood. It is noteworthy that one bipolar husband also developed a post-partum mania, emphasizing the importance of stress as well as post-partum physiology. This observation prompted a retrospective chart review and further data collection to test the hypothesis that in a population of male manic patients, episodes of post-partum psychoses occur in proximity to births of children.

The six male infants born to these couples are participating with their parents in a study of the development of infants at risk for bipolar illness. These children are matched with controls, both of whose parents are free of any psychopathology. Each infant is studied on three occasions up to the age of 20 months. The studies include measurement of development, temperament and affect, a neurological examination, average evoked response testing, a study of parental attitudes and behaviors, and a detailed family history with an emphasis on family psychodynamics. An analysis of video tapes of an infant stress paradigm is done by investigators at the University of Colorado.

In addition, a graduate nurse has collected data from the clinic this past year for her doctoral thesis entitled, "Predictors of Compliance in Patients Being Treated for Manic-Depressive Psychosis in an Outpatient Clinic".

Our collaboration with Dr. Paul Grof focusing mainly on an analysis of the course of unipolar and bipolar illness in 334 patients studied without maintenance treatment, has continued this past year. The unipolar/bipolar dichotomy is based on the presence or absence of mania during the course of the illness. However, the first manic episode may not occur until late in the course of the illness as already pointed out by Perris. In our sample 15% of all bipolar patients did not switch into mania until after the third episode of the illness (false unipolar). Early discrimination between a unipolar or bipolar diagnosis is of critical importance for treatment as well as for interpretation of research findings. Although bipolar and false unipolar patients tend to have an earlier age of onset than "true" unipolar patients, such features as the length of the first cycle do not differentiate these groups. Furthermore, although some authors suggest that one criterion for unipolar illness should be the occurrence of three or more depressive episodes, our analysis suggests that this criterion adds almost nothing to the specificity of the diagnosis, but may well sacrifice the representativeness of the unipolar sample.

In both the unipolar and bipolar groups age of onset was positively correlated (r = +0.5) with the time between the first and second episode. For example, in bipolar patients with an age of onset below 30, the chance of the second episode occurring within 24 months is only about 20%, whereas with an age of onset in the 30's, the chances of another episode within 24 months exceeds 50%. For the very late age of onset group (50's and 60's), the 24 months relapse probability is greater than 80%.

Publications:

Davenport, Y.B., Adland, M.L., Gold, P.W., and Goodwin, F.K.: Manic-depressive illness: psychodynamic features of multigenerational families. Amer. J. Orthopsychiat. 49(1): 24-35, 1979.

Targum, S.D., Davenport, Y.B., and Webster, M.V.: Post-partum mania in bipolar manic-depressive patients withdrawn from lithium carbonate. J. Nerv. Mental Dis. (in press).

Targum, S.D., Dibble, E.D., Davenport, Y.B., and Gershon, E.S.: Etiology, familial risk, and burden of bipolar illness: perceptions of patients and spouses. Arch. Gen. Psychiat. (in press).

Zis, A.P., Grof, P., and Goodwin, F.K.: The natural course of affective disorders: implications for lithium prophylaxis. In Cooper, T., Gershon, S., Kline, N.S., and Schou, M.: Lithium: Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, in press.

Zis, A.P., Grof, P., Webster, M., and Goodwin, F.K.: Prediction of relapse in recurrent affective disorder. Psychopharm. Bull. (in press).

Zis, A.P. and Goodwin, F.K.: Major affective disorder as a recurrent illness: a critical review. Arch. Gen. Psychiat. 36(8): 835-839, 1979.

Jamison, K.K., Gerner, R.H., and Goodwin, F.K.: Patient and physician attitudes toward lithium: relationship to compliance. Arch. Gen. Psychiat. 36(8): 866-869.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01852-01 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Brief Outpatient Studies of Depressive Syndromes and Character Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
OTHER:	Frederick Goodwin	Chief	CP/NIMH
	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
	Gay Grover	Clinical Nurse Expert	CC Nursing
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Steven Paul	Chief, Unit on Preclinical Pharm.	CP/NIMH
	Ed Silberman	Clinical Associate	LPP/NIMH
	Herbert Weingartner	Acting Chief	LPP/NIMH
	Monte Buchsbaum	Chief, Section on Clin. Psychopharm.	BP/NIMH
	Dennis Murphy	Chief	CN/NIMH
	Thomas Wise	Chairman, Dept. of Psychiatry	Fairfax Hosp.
	John Nardini	Private Practice	
	David Charney	Private Practice	

COOPERATING UNITS (if any)
Section on Clinical Psychophysiology, BP; CN; Fairfax Hospital Dept. of Psychiatry; Private practitioners in the Washington metropolitan area.

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INSTITUTE AND LOCATION
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the great majority of patients with depression-spectrum illnesses are successfully treated outside the hospital, an outpatient facility for clinical and biological evaluation and treatment is essential, both to help assure appropriate clinical care and to develop an adequate and representative sample of affectively-ill patients for study. The clinic is developing a collaborative/consultative model, whereby community-based practitioners can refer patients for evaluation and yet continue to treat the patients themselves. A pilot project in the evaluation and treatment of unipolar depression has been conducted this past year, and will now be focused on exploring clinical and biological predictors of monoamine oxidase inhibitor response. Plans for studies of the borderline syndromes (neurophysiology and pharmacotherapy), and premenstrual and postpartum affective syndromes (neuroendocrinology and psychopharmacology) are in the planning stages.

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Richard Schaengold	Private Practice

Project Description:

This year marked the beginning of a pilot project in the Outpatient Clinic, CPB, that explores the feasibility of collaborative/consultative research and treatment programs involving outpatients referred by private practice psychiatrists in the Washington, D.C. area. The initial project provided for a five-day outpatient evaluation of depressed patients referred by several area psychiatrists, followed by a double-blind "drug-of-choice" treatment phase if agreed to by the treating physician, the patient, and the clinic staff. Several conclusions can be drawn from our experience with the first ten patients. First, the program has been of significant benefit to all but one of the patients referred. Second, the patients are a more representative sample of the spectrum of depressed patients than those referred for inpatient evaluation and treatment. Third, there are major advantages to the collaborative model, since private psychiatrists can continue to provide psychotherapy and continuity of care (thus benefitting the patient), and since this model encourages the development of an ongoing relationship between the referring psychiatrist and the clinic staff.

The project includes the collection of information on the course of the illness, symptomatology, psychological testing, physical and neurological examination, cortical evoked response patterns, urinary and cerebrospinal fluid metabolites, and the response to a single dose of dextroamphetamine. A variety of treatment approaches have been used. In the coming year, the project will continue, but will focus on clinical and biological findings which may predict response to monoamine oxidase inhibitors.

The availability of a full-time position for the Chief, Outpatient Unit, will enable the clinic to embark on a series of outpatient studies of other disorders as well. The Unit will provide a means of following rapid cycling bipolar patients after an initial inpatient stage, trying to find a satisfactory treatment for this often treatment-refractory syndrome. Premenstrual and postpartum affective syndromes will be investigated, in terms of neuroendocrine function and response to psychopharmacologic agents. Finally, the neurophysiology of selected aspects of the borderline syndrome will be examined collaboratively with the Section on Clinical Psychophysiology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01831-03 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Basic and Clinical Studies of the Neuronal and Glial Enolases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. Marangos	Chief, Unit on Neurochem.	CP/NIMH
OTHER:	Frederick K. Goodwin	Chief	CP/NIMH
	A. P. Zis	Guest Worker	CP/NIMH
	Milton Brightman	Head, Sec. on Neurocytology	LNNS/NINCDS
	Donald Schmechel	Medical Officer	LCS/NIMH
	C. Schengrund	Assoc. Prof. Biochem.	Hershey Med.
	B. Trapp	Research Associate	LNNS/NINCDS
	A.G.E. Pearse	Professor	Royal Med. Sch. (London)
	J. M. Polak	Neuroanatomist	Royal Med. Sch. (London)
	B. Festoff	Chief of Neurology	VAH KC
	I. Campbell	Visiting Scientist	CN/NIMH

COOPERATING UNITS (if any) Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS; Laboratory of Clinical Science, NIMH; Hershey Medical Center; Royal Medical School, London; VA Hospital, Kansas City; Clinical Neuropharmacology, NIMH; Medical Neurology, NINCDS; Western General Hospital, Scotland; Johns

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Specific biochemical markers for the two major cell types of the nervous system have been determined. These proteins are isoenzymes of the glycolytic enzyme enolase and are specifically Neuron Specific Enolase (NSE) and Non-Neuronal Enolase (NNE). NSE is present in all types of neurons and NNE is localized in glial cells within the nervous system. Studies are currently directed at elucidating the physiological significance of these two structurally, functionally, and immunologically distinct proteins, and their utilization as specific markers in probing the functional organization of brain. Recent immunocytochemical findings have shown that during neuronal maturation NSE is acquired gradually, and a switch from NNE to NSE occurs. NSE has also been demonstrated in peripheral neuroendocrine cells of the APUD category. The very recent development of a more sensitive radioimmunoassay has made possible clinical studies where levels of NSE and NNE in serum and cerebrospinal fluid will be correlated with neuronal and glial pathologies respectively.

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

V. Askanas	Pathologist	MN/NINCDS
F. L. Majors	Staff Psychiatrist	CN/NIMH
D.J.H. Brock	Research Neurologist	Western Gen. Hosp., Scotland
S. Snyder	Professor	Johns Hopkins
A. J. Dutka	Associate Neurologist	NNMC
R.E. Fellows	Chairman, Dept. of Physiology & Biophysiology	U. of Iowa
H. Koppel	Neuroanatomist	Royal Med. Sch. (London)
B. Winblad	Pathologist	UMEA (Sweden)
D. Murphy	Chief	CN/NIMH
Vanda Lennon	Immunopathologist	Mayo Clinic

Cooperating Units (if any) continued:

Hopkins University Medical Center; National Naval Medical Center; UMEA, Sweden; Mayo Clinic, Rochester, Minnesota

Project Description:

The structural complexity and functional diversity of nervous tissue makes the study of brain function a very difficult task. It is often impossible to account for the many variable parameters in the study of the molecular mechanisms of brain function that are largely due to the many different cell types and their elaborate interconnections. For this reason, a concentrated effort has been directed toward developing cell culture systems where neurons and glia can be individually studied. A related approach, taken in our laboratory, involves the molecular characterization of protein that are specific to neuronal or glial cells. The assumption is that these proteins would have specific functions necessary to the physiology of each respective cell type and that their characterization will provide basic insights concerning the molecular biology of each cell type. Such studies have the potential of expanding our basic knowledge of neuronal and glial interrelationships.

Previous studies from our laboratory have shown that NNE and NSE are structurally, functionally and immunologically distinct. NSE from rat and human brain is a dimer of two identical 39,000 Dalton subunits designated $\gamma\gamma$, whereas NNE is a dimer of two identical 43,500 Dalton subunits designated $\alpha\alpha$. NSE is acidic while NNE is neutral. Antisera raised against NSE do not react at all with NNE and vice versa, indicative that the two proteins are immunologically unrelated. These data clearly show that NNE and NSE are separate gene products. Functional studies have also shown a marked stability of NSE towards chloride salts whereas NNE is quite labile under these conditions. This is consistent with the physiology of the neuron, since chloride concentrations can become quite high and the lack of a specialized form of enolase would result in inactivation and the consequent blockage of glycolysis and the TCA cycle. This would result in decreased ATP and rapid neuronal cell death.

During the past year we studied the correlation between NSE and the state of neuronal differentiation. Using both immunocytochemical staining methods and the specific radioimmunoassays (RIA) we have shown that undifferentiated neurons contain NNE and undergo a switch over to NSE in a manner that is coincident with differentiation. This finding is of great importance, since it indicates that NSE is not only a neuronal marker but is specific to differentiated, functional neurons, and will therefore be of considerable value to developmental neurobiologists. Immunocytochemical studies are currently in progress attempting to determine the specific developmental processes to which the NNE-NSE switch is coupled. This will provide potentially valuable information concerning the specific metabolic adaptations necessary for neuronal function.

The demonstration in this laboratory that NSE is also present in peripheral neuroendocrine cells of the APUD series has lent considerable support to the hypothesis formulated by A.G.E. Pearse that these cells are "neuron-like" and constitute a separate division of the nervous system. We are now involved in a major collaborative effort with Dr. Pearse and his associate Dr. Julia Polak, directed at identifying presumptive neuroendocrine cells in the gut. Immunocytochemical and radioimmunological determination of NSE offers a very simple and powerful technique for identifying and characterizing these cells and many other peripheral cell types. Additionally an interesting question concerns metabolic demands that may be common to neurons and neuroendocrine cells that require their both having this unique glycolytic enzyme.

Extensive collaborative studies are also underway involving primary neuronal cell cultures. We have shown substantial levels of NSE in cultured mouse spinal cord neurons (with Dr. Jeffery Barker), cultured rat cerebral neurons (with Dr. Cara Schengrund) and brain aggregate cultures (with Dr. Bruce Trapp) by both RIA and immunocytochemistry. Developmental studies in the last two systems have just been completed with the results supporting the whole animal studies, i.e., NSE is correlated with differentiation. In the brain aggregate system, the NNE to NSE switch has also been directly demonstrated by immunocytochemical staining. The studies have established these culture systems as being representative of nervous tissue in vivo, which makes possible their use as model systems for nervous tissue. Studies are being planned to determine the effects of neuronal stimulation on NSE levels as well as the relationship between cell age and the protein.

During the last year a new double antibody RIA has been developed for NNE and NSE from rat, monkey and human brain. Using these assays the regional distribution of both proteins has been determined in monkey and rat brain. Areas high in white matter, such as the corpus callosum and lower brain stem, showed high NNE/NSE ratios, whereas rich grey matter areas had low ratios. In general the spinal cord had lower NSE levels than brain, and the peripheral nervous system levels were the lowest. NNE/NSE ratios of 1-4 are found in brain, 3-5 in spinal cord and 10-20 in peripheral nervous tissue such as dorsal root ganglia and various nerves. The wide range of NSE levels compared to NNE suggests that neuronal enolase levels are correlated with the functional state of the neuron. Three cell classifications have emerged based on enolase isoenzyme content: 1) neurons that contain only NSE; 2) neuroendocrine cells that contain both NSE and NNE; and 3) non-nervous tissue that does not contain NSE.

The blood platelet has been used as a model system for the monoaminergic neuron based on its ability to handle amines in a similar manner to neurons. During the past year we have shown that human blood platelets contain very significant levels of NSE. This provides the first evidence that platelets are similar to neurons with respect to a neuron specific macromolecule, and further supports their use as a neuronal model system. This finding raises questions concerning the embryologic origin of the platelet and specifically whether it can be considered a member of the APUD classification.

Kainic acid is a recently described GABA analogue that has been reported to have neurotoxic effects. Injection of kainic acid into the striatum of rat leads to a virtual destruction of neuronal cell bodies while leaving axons of passage virtually unaffected. This system was assessed biochemically by determining the effect of kainic acid on the levels of NNE and NSE at various times after injection. The results showed 50% to 60% decreases in NSE and a small but not significant decrease in NNE at 9 days post injection. These results are consistent with the distribution of NSE and with the postulated effects of this neurotoxic agent.

During the past year an effort has been made to use the NNE and NSE assays in clinical studies. Specifically, levels of NNE and NSE in CSF or serum are being evaluated as markers of glial or neuronal pathologies respectively. An ^{125}iodine assay that is 15-20 fold more sensitive than the tritium procedure has been developed. Use of this assay makes possible the determination of levels of NSE in the CSF, and at present a number of collaborative studies involving neurological and stroke patients have been initiated. Preliminary results in stroke patients indicate that NSE levels rise at some time after the stroke. At present the exact time course of tissue destruction and NSE release into the CSF is not known. Studies are in progress using monkeys with lumbar cannulas. In these studies monkeys are made alcoholic and evidence of brain damage is sought by assaying for NSE. These studies are not yet complete.

Extensive studies were started in collaboration with Dr. B. Winblad concerning levels of NSE and NNE in various areas of senile dementia brain tissue, obtained at autopsy. In these studies 23 brain areas will be analyzed in various types of dementias and compared to age matched controls. The effects of normal aging on NNE and NSE levels will also be determined.

Preliminary studies done in collaboration with Dr. Brock have shown that high NSE levels in amniotic fluid of pregnant women may be predictive of neurological defects in the fetus. In the small number of amniotic fluids we have analyzed to date (12 cases), elevated NSE levels were found in anencephalic and spina bifida cases. Further studies indicate that NSE levels in aminiotic fluid may provide an early presumptive diagnosis for certain birth defects of a neurologic nature.

Various purified neuronal and glial cell populations are being used to determine the cellular localization of various receptors. Collaborative studies are currently in progress with Dr. S. Snyder using the NNE and NSE methodology to determine the purity of these various cell fractions. Morphological criteria are not very useful since the cellular preparation procedure destroys much of the cyto-architecture. Synaptosomal preparations are also being analyzed with preliminary results indicating that a large portion of this cellular subfraction, which has traditionally been thought to be neuronal, is actually glial in nature.

The enolase system has recently become of interest to a large group of neuroscientists ranging from neuroanatomists to neurologists and neurosurgeons. As a service our laboratory has supplied antisera to numerous groups throughout the country and the world for the purpose of identifying cell types in various primary and transformed biopsy tissue. It appears likely that cytochemical analysis for NSE by immunocytochemistry or RIA will become an important technique for neuropathologists in identifying neurons or neuron-like cells in tissue. This situation has in large part been responsible for the recent commercial interest in the enolase methodology. Specifically The New England

Nuclear Corporation has approached us in regard to their making a RIA kit for the enolases as well as marketing the antisera for direct immunocytochemical studies. The availability of such an assay kit and the antisera will stimulate work in many areas of neurobiology as well as relieve us from our current role as the supplier of these reagents.

Publications:

Schmechel, D., Marangos, P.J., and Brightman, M.: Neuron specific enolase: a molecular marker for neuroendocrine cells. Nature 276: 834-836, 1978.

Marangos, P.J., Parma, A.M., and Goodwin, F.K.: Functional properties of neuronal and glial isoenzymes of the glycolytic enzyme enolase in brain. J. Neurochem. 31: 727-732, 1978.

Marangos, P.J., Zis, A.P., Clark, R.L. and Goodwin, F.K.: Neuronal (NSE), non-neuronal (NNE), and hybrid forms of enolase in brain: structural, immunological, and functional comparison. Brain Res. 150: 117-133, 1978.

Marangos, P.J., Schmechel, D., Zis, A.P. and Goodwin, F.K.: The existence and neurobiological significance of neuronal and glial forms of the glycolytic enzyme enolase. Biol. Psychiat. 14: 563-579, 1979.

Marangos, P.J. and Schmechel, D.: The neurobiology of the brain enolases. In Youdim, M.B.H., Sharman, D.F., Lovenberg, W. and Lagnado, J.R. (Eds.): Essays In Neurochemistry and Neuropharmacology. New York, John Wiley & Sons, 1979, in press.

Marangos, P.J., Schmechel, D., Parma, A.M., Clark, R.L., and Goodwin, F.K.: Measurement of neuronal and non-neuronal enolase in rat, monkey, and human tissues. J. Neurochem. (in press).

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Schmechel, D., Brightman, M.W., and Marangos, P.J.: Neurons switch from non-neuronal (NNE) enolase to neuronal (NSE) enolase during development. Brain Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01836-01 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
C.N.S. Benzodiazepine Receptors: Physiological and Pharmacological Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Frederick Goodwin	Chief	CP/NIMH
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	Phil Skolnick	Pharmacologist	LBC/NIAMDD
	Peter Syapin	Asst. Professor, Dept. of Neurology	USC Sch. of Medicine
	John Tallman	Pharmacologist	APB/NIMH
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	John MacDonald	Visiting Scientist	LNP/NINCDS
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COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIAMDD; Laboratory of Neurophysiology, NINCDS; Laboratory of Chemistry, NIAMDD; Laboratory of Clinical Science, NIMH; Laboratory of Preclinical Studies, NIAAA; Dept. of Neurology, University of Southern California; Dept. of Physiology, University of Texas;

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1.25	0.70	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The identification of saturable, high affinity, and stereospecific binding sites for benzodiazepines has been described in the mammalian central nervous system. The correlations between the binding of a series of benzodiazepines in vitro and their pharmacologic potencies as anxiolytics, anticonvulsants, and muscle relaxants in vivo, strongly suggest these sites are receptors mediating the therapeutic actions of the benzodiazepines. The physiologic role(s) of the benzodiazepine receptor and the pharmacologic, behavioral, and physiologic regulation of this receptor are being investigated using several experimental paradigms, including: 1) the isolation and identification and pharmacologic assessment of endogenous substrates which subserve this receptor (see Project No. Z01 MH 01834-02); 2) development of suitable in vitro models for studying the benzodiazepine receptor such as primary and transformed cultures of neural origin and mammalian retina; 3) development of novel benzodiazepines to characterize receptor subgroups and to determine the physiologic function of these subgroups; 4) development of an irreversible ligand to the benzodiazepine receptor for studying receptor turnover; 5) development of suitable techniques for studying benzodiazepine receptors in vivo.

PHS-6040
(Rev. 10-76)

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

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Ronald Schoenfeld	Guest Scientist	CP/NIMH
Michael Ebert	Medical Officer	LCS/NIMH
Evan Williams	Visiting Fellow	LBC/NIAMDA
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Cooperating Units (if any) continued:

Section on Biochemistry, ABP, NIMH

Project Description:

Objectives: To characterize and assess the physiologic role(s) of the benzodiazepine receptor in the mammalian central nervous system and to develop suitable in vitro model systems. To develop novel compounds useful in the characterization of these receptors and define the physiologic role and pharmacologic function of the receptor. To assess the importance of the benzodiazepine receptor in adaptive changes following physiologic, pharmacologic, and/or behavioral changes.

Methods Employed: Neurochemical characteristics of the benzodiazepine receptor were studied with a radioreceptor assay using tritium labeled benzodiazepines. In vivo pharmacologic testing of compounds which bind to the receptor in vivo was accomplished using intraventricular injection and peripheral injection of standard convulsants. Environmental manipulation, electroconvulsive shock and chemical intervention in vitro have been used to determine the importance of the receptor in maintaining physiologic and behavioral homeostasis and in determining the pharmacologic importance of these receptors.

Major Findings: The development of an in vivo method for labelling benzodiazepine receptors has been accomplished. Specific binding of radioactive benzodiazepine to brain was demonstrated under physiological conditions in vivo. The regional and subcellular distribution of benzodiazepine binding in vivo shows a near perfect rank-order correlation with binding obtained in vitro. This method should be useful in demonstrating drug/behavioral-induced changes in the benzodiazepine receptor in vivo.

The importance of benzodiazepine receptors in mediating pentylenetetrazol-induced seizures was examined in mice. A high correlation was demonstrated between the number of "diazepam-occupied" receptors following anticonvulsant doses of diazepam and the number of animals protected against pentylenetetrazol-induced seizures. Furthermore, no more than thirty percent of the receptors need be occupied to manifest fully anticonvulsant activity. This is the first study directly relating occupation of a central receptor site with the clinical effects of a drug.

The role of the benzodiazepine receptor in mediating seizure activity was studied using two seizure models: pentylenetetrazol-induced convulsions and electroshock. A rapid increase in the number, but not affinity of benzodiazepine receptors was observed following tonic-clonic convulsions induced in both these models. This increase was fully manifest 15 minutes post-ictal and was no longer apparent 60 minutes post-ictal.

A simple and sensitive radioreceptor assay for benzodiazepines in plasma and other biological tissues was developed. The sensitivity obtained is equal to or greater than currently used techniques of gas-chromatography with electron capture detection. In addition, the radioreceptor technique is rapid, requires no expensive equipment other than that available in most clinical laboratories, and gives a measure of the therapeutic equivalents of benzodiazepine that can be compared directly with diazepam. Patent rights for this technique are pending (issued to Drs. Paul and Skolnick through NIH).

Various models for studying the benzodiazepine receptor have been investigated, including mammalian retina, transformed cells of neural origin, and primary cultures of fetal brain and spinal cord. Data obtained from these models suggests that both the retina and primary cultures of brain and spinal cord may be used to study "brain specific" receptors in culture in order to determine the physiologic and pharmacologic function(s) of this receptor.

Irazepine, a noncompetitive, irreversible inhibitor of benzodiazepine binding is the first in a series of new synthetic compounds that will be utilized to investigate the structure and biochemical characteristics of the receptor.

The effects of alcohol dependence and withdrawal from alcohol on the benzodiazepine receptor have been examined. Small but significant reductions in benzodiazepine receptor number have been demonstrated in animals undergoing alcohol-induced seizures.

Recent studies have been designed to examine the role of depolarizing stimulation on benzodiazepine receptors. In addition, the effects of stress-related hormones such as corticosterone and ACTH will be examined as possible modulators of the benzodiazepine receptors. The development of an in vitro system for studying benzodiazepine receptor function has been initiated.

Significance to Biomedical Research: The benzodiazepines are the most widely prescribed drugs in current therapeutic use. Understanding their mechanism of action is of fundamental importance to the understanding of diseases such as epilepsy, anxiety-neuroses, and alcoholism, as well as development of a more fundamental understanding of brain function. In addition, these studies may provide information regarding the development of therapeutic agents superior to the benzodiazepines.

Proposed Course of Project: The importance of the benzodiazepine receptor in various disease models (e.g., epilepsy) will be studied. Alterations in benzodiazepine receptors following two seizure models pentylenetetrazole and electroconvulsive shock will be expanded to determine if interaction with the benzodiazepine receptor is a universal first step in the generation and prevention of seizures. The alterations in benzodiazepine receptors following changes in chemical, pharmacologic, or physiologic environment in cultured models will be examined. Irreversible inhibitors of benzodiazepine receptors will be used to attempt to measure receptor turnover in vivo, isolate the benzodiazepine receptor, and characterize receptor subtypes. Further identification of brain and pituitary inhibitors of benzodiazepine binding will be attempted, and their subsequent testing in the in vitro systems developed this past year will be carried out. The presence of "benzodiazepine-like" substances in human and monkey cerebrospinal fluid will be studied using the inhibition of receptor binding as a screening technique.

Publications:

Paul, S.M. and Skolnick, P.: Acute changes in brain benzodiazepine receptors following experimental seizures. Science 202: 892-894, 1978.

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Skolnick, P., Syapin, P., Paugh, B., and Paul, S.: Reduction in benzodiazepine receptors associated with Purkinje cell degeneration in "nervous" mutant mice. Nature 277: 397-399, 1979.

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Williamson, M., Paul, S.M., and Skolnick, P.: Demonstration of [^{3}H] diazepam binding to benzodiazepine receptors *in vivo*. Life Sci. 23: 1935-1940, 1978.

Skolnick, P., Goodwin, F., and Paul, S.: A simple and sensitive radioreceptor assay for plasma benzodiazepines. Arch. Gen. Psychiat. 36: 78-80, 1979.

Paul, S.M., Marangos, P.J., Skolnick, P. and Goodwin, F.K.: Brain specific benzodiazepine receptors and putative endogenous "benzodiazepine-like" compounds. Psychopharm. Bull. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01834-02 CP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Endogenous Ligands for the Brain Benzodiazepine Receptor

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stereospecific, high affinity, saturable receptor sites in brain for the benzodiazepines suggests that at least some of their pharmacologic actions are receptor mediated. Investigations in our laboratory have centered on the characterization of physiologically relevant ligands for the benzodiazepine receptor, with the ultimate goal of better understanding the molecular mechanisms of anxiety mediation in the central nervous system. Studies from our laboratory have shown that the purines which include compounds such as inosine, hypoxanthine, guanosine, etc., all specifically inhibit $[^3H]$ diazepam binding to rat brain synaptosomal membranes. The methyl xanthine stimulants caffeine and theophylline also inhibit diazepam binding, suggesting that their mechanism of action involves the benzodiazepine receptor. Evidence for the purines being physiologically relevant ligands comes from recent studies showing antiseizure activity of the purines and direct neurophysiological actions of the purines when iontophoresed onto cultured neurons. Further biochemical and behavioral studies are in progress involving chronic purine administration, and cyclic AMP coupling systems. Studies in our laboratory have also identified two additional factors which inhibit diazepam binding and which do not appear to be purines. The identity of these compounds is currently being investigated.

Project Description:

The recent discovery of a specific receptor for the benzodiazepines in brain has prompted intensive studies in our laboratory aimed at the elucidation of naturally occurring "benzodiazepine-like" compounds. Since the affinity of a series of benzodiazepines for the receptor is highly correlated with their pharmacologic potency, it is likely that endogenous ligands will serve to modulate or mediate these effects (i.e., anxiolytic, anticonvulsant and muscle relaxant effects). Therefore, it seems that the biochemical and pharmacologic characterization of such compounds would greatly expand our conceptions concerning the biological mechanisms of these effects. Questions that emerge are: (1) Is there an anxiety mediating system in brain? (2) Do naturally occurring anxiolytic and anxiety-causing substances exist, and if so, by what mechanism do they act? (3) Are seizures mediated by the benzodiazepine receptor?

Studies done in our laboratory have resulted in the isolation and characterization of endogenous inhibitors of specific [^{3}H] diazepam binding from bovine brain. These substances have been identified as the purines inosine and hypoxanthine. The structurally related pyrmidines are totally inactive as inhibitors of diazepam binding. We have shown that a broad spectrum of purines inhibit diazepam binding and have established some structure-activity relationships. The 2-deoxypurines are three to four fold more potent than their hydroxylated counterparts (i.e., 2-deoxyinosine and 2-deoxyguanosine) in their inhibition of diazepam binding. Methylation of the purine nucleus in the 7 position abolishes inhibitory activity, whereas methylation in the 1 position increases potency. Phosphorylated purines such as AMP, ADP, ATP, ITP and GTP are not active as is the case for the cyclic nucleotide phosphates. Purines are specific inhibitors of diazepam binding and have no effect on other receptor-ligand systems (i.e., opiate, adrenergic, cholinergic). It therefore seems likely that only the benzodiazepines and the purines can serve as ligands for the receptor. The multiplicity of active purines raises questions as to which purine(s) are physiologically relevant.

Since the common methyl-xanthine stimulants caffeine, theophylline and theobromine are methylated purines, these compounds were tested for binding inhibitory activity. All of these compounds competitively inhibit diazepam binding and more interestingly, their relative potencies directly parallel their potencies as cortical stimulants (caffeine > theophylline >theobromine). The convulsant pentylenetetrazol (PTZ) also competitively inhibits diazepam binding. Since all these compounds are convulsants at high doses, and diazepam is an anticonvulsant, the implication is that seizures are mediated by the benzodiazepine receptor. It is possible that the xanthine stimulants and PTZ are agonists and diazepam the antagonist, at least as concerns seizures. These questions are currently under investigation using pharmacologic and neurophysiologic techniques.

Behavioral and neurophysiological studies have been done in order to determine whether the purines have "benzodiazepine-like" physiological effects. It has been shown that intracerebroventricular administration of inosine more than doubles PTZ-induced seizure latency. This is a benzodiazepine-like effect, since diazepam inhibits PTZ-induced seizures. The 7-methyl inosine derivative that is ineffective in inhibiting diazepam binding also has no effect on seizure latency, whereas 2-deoxyinosine, a very potent inhibitor of diazepam binding, increases seizure latency three to four fold. The purines do not completely protect against seizures as does diazepam. This is probably due to their intimate association with intermediary metabolism. The purines have therefore been shown to be "benzodiazepine-like" with regard to the parameter of PTZ-induced seizures, and differ only in that they are short acting anti-seizure compounds.

Neurophysiological experiments performed in collaboration with Dr. Jeffery Barker have shown that direct iontophoretic application of inosine on cultured mouse spinal cord neurons activates an inhibitory and an excitatory conductance mechanism. Furthermore, the excitatory response was cross desensitized by flurazepam, indicating that inosine and flurazepam were acting at the same site. Again, this result indicates that inosine has benzodiazepine-like effects. Further neurophysiologic characterization of other purines and the methyl xanthines will provide important information concerning possible agonist, antagonist relationships.

Experiments are presently in progress attempting to characterize further the relationship between purines and the benzodiazepine receptor. Due to the high levels of purines required, their rapid metabolism, and poor permeability to the blood-brain barrier, pharmacological experiments are difficult to design. Therefore, we have chosen oral administration of various purines to mice. Animals are currently being maintained on a purine-free diet, and their diets supplemented with inosine or caffeine or adenosine. These animals will be studied behaviorally, pharmacologically and biochemically. For example, the number and affinity of their brain benzodiazepine receptors will be assessed as will their threshold for PTZ-induced seizures and their conflict avoidance behavior. Results of these studies may show dietary factors to have effects on the mediation of anxiety and seizure activity, as well as provide important information on the behavioral and physiological effects of these compounds.

Studies are also in progress attempting to demonstrate a biochemical correlate of benzodiazepine receptor stimulation. For example, can an effector process be shown, such as an increase in cyclic AMP? If this can be demonstrated and the purines shown to have properties similar to the benzodiazepines, it would provide important evidence supporting these endogenous compounds as being linked to a physiological effector mechanism.

It would be important to demonstrate direct binding of labelled purines to synaptosomal membranes and thus, lend further support to the hypothesis that these compounds are ligands for the benzodiazepine receptor. We proceeded with these experiments despite the fact that the purines' low affinity binding would be difficult, if not impossible, to demonstrate. [^{3}H] 2-deoxyguanosine, [^{3}H] caffeine and [^{3}H] 2-deoxyadenosine binding have been demonstrated. Only 30-40% of the binding is displaced by unlabelled purine. Diazepam inhibits binding to an even lesser extent. Methodological difficulties encountered include extremely low bound/free ratios. In spite of these difficulties, experiments are continuing in an effort to show benzodiazepine displaceable purine binding.

The purines were extracted from brain tissue using acidified acetone. Since these conditions may have excluded active compounds, an aqueous extraction procedure was tried yielding four active diazepam binding inhibitory fractions--inosine, hypoxanthine, and two larger factors of unknown identity. These two factors have been designated peak I and peak II. Both are heat stable and competitively inhibit $[^3H]$ diazepam binding. Peak I has a molecular weight of about 1000 and peak II about 500. Peak I is only found in brain and pituitary, whereas peak II is also found in muscle and liver. Further purification and characterization of these compounds will reveal whether they are physiologically relevant endogenous ligands for the benzodiazepine receptor. It seems, therefore, that there are multiple, naturally occurring "benzodiazepine-like" compounds in brain. This is similar to the endogenous opioid system that has also been shown to contain multiple ligands.

Work is also in progress aimed at better understanding the physiology of the benzodiazepine receptor itself. Specifically studies are being done attempting to characterize the circadian pattern of receptor number and affinity. Preliminary results have shown that in cerebral cortex the receptor number increases significantly at 10 a.m. No change in receptor affinity is observed. In the striatum the circadian rhythm is quite different with the highest levels in the evenings. Also, the antidepressant, imipramine, shifts the striatal rhythm while having no effect on the cortical profile. The striatal benzodiazepine rhythm and its alteration by imipramine is identical to that observed for the dopamine receptor system, suggesting that these two systems may be coupled in the striatum.

Studies are in progress in collaboration with Dr. Winblad of UMEA using human post mortem tissue. In these studies benzodiazepine receptor status will be assessed in various neurological conditions, such as senile dementia and Parkinson's disease. The effects of age in normal tissue is also being examined. This very valuable tissue will also be used to determine whether any laterality differences exist in the benzodiazepine receptor. These results will be compared with data on the dopaminergic, γ and β adrenergic and cholinergic receptors, and should therefore provide a comprehensive assessment of receptor status in normal and senile dementia human brain.

Publications:

Skolnick, P., Syapin, P.J., Paugh, B.A., Moncada, V., Marangos, P.J., and Paul, S.M.: Endogenous inhibitor of ^{3}H diazepam binding alters pentylenetetrazol evoked seizure latency. PNAS 76: 1515-1518, 1979.

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Skolnick, P., Marangos, P.J., Syapin, P., Goodwin, F.K., and Paul, S.: CNS benzodiazepine receptors: physiological studies and putative endogenous ligands. Pharmacology, Biochemistry and Behavior 10: 815-823, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01837-01 CP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Endocrine Effects on C.N.S. Neurotransmitters and Receptors

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TOTAL MANYEARS: 0.28	PROFESSIONAL: 0.50	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The behavioral and neuroendocrine effects of various peripheral hormones, including the gonadal steriods, thyroid hormone(s), and anterior pituitary hormones, have been described by many investigators. Nevertheless little data exists on the precise neurochemical mechanisms mediating hormone-CNS interactions. Furthermore, despite the thorough characterization of hormone receptor(s) in the CNS over the past 10 years, there is virtually no understanding of how hormones interact physiologically with neurons. Studies in our laboratory have been designed to study the biochemical effects of hormones on neuronal preparations in vitro in an attempt to identify direct neurochemical effects on excitable tissue. Studies thus far have shown significant effects of biologically active estrogens on catecholamine efflux from the isolated hypothalamus in vitro. These effects were concentration dependent and not observed with biologically inactive estrogens such as estradiol-17α. These results suggest that estrogen may facilitate the release of catecholamines from the hypothalamus.

Project Description:

Objectives: To study the neurochemical effects of various peripheral hormones on neurotransmitter-related activity, including transmitter release, turnover, and post-synaptic receptor sensitivity. This project was initiated last year in the Laboratory of Clinical Science, NIMH.

Methods Used: Radioreceptor assays, organ and tissue culture, radioenzymatic assays for neurotransmitters, radioimmunoassay of cyclic nucleotide and hormones.

Major Findings: Incubation of hypothalamic explants with the potent non-steroidal estrogen, diethyestilbestiol (DES) resulted in a significant increase in norepinephrine efflux during a 70 min. incubation. The time course and dose-response characteristics of the DES-induced efflux of norepinephrine were similar to effects of estrogen on cyclic AMP accumulation in the same tissue preparation. Studies of the regional distribution of this release within the hypothalamus indicate a marked correlation with the concentration of estrogen receptors, suggesting that catecholamine efflux may be related to an estrogen-receptor interaction.

Significance to Biomedical Research: An understanding of the neurochemical mediators of hormone action within the CNS will have broad implications to a number of disease states in man. Additionally, since a number of hormones have been reported to have behavioral and mood effects, and since abnormal endocrine homeostasis may be involved in the pathophysiology of affective illness, the precise neurochemical events mediating hormone action may be helpful in elucidating neurochemical substrates in depression.

Proposed Course of Project: The present studies will be expanded to include a number of other peripheral hormones, including prolactin, thyroxine, ACTH, α-MSH, progesterone, and corticosterone. The effects of these hormones on neurotransmitter levels and turnover will be coupled with possible changes in neurotransmitter receptors.

Publications:

Paul, S.M., Axelrod, J., Saavedra, J., and Skolnick, P.: Estrogen-dependent efflux of endogenous catecholamines from the hypothalamus. Brain Res. (in press).

Paul, S.M., Axelrod, J., and Skolnick, P.: Estrogen dependent efflux of endogenous catecholamines from the hypothalamus. In Usdin, E., Kopin, I., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, Vol. II, pp. 1206-1208.

Paul, S.M. and Axelrod, J.: Catechol estrogens: Localization, formation, and actions in brain and other estrogen-sensitive tissues. In DiCarlo, F. (Ed.): Pharmacological Modulation of Steroid Action. New York, Raven Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00422-08 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Neuropharmacology of Circadian Rhythms and the Effects of Light and Darkness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Medical Officer (Research)	LCS NIMH
Others:	M. Brownstein, Head, Unit on Neuroendocrinology	LCS NIMH
	D. Jimerson, Senior Staff Fellow	LCS NIMH
	M. Herkenham	LNP NIMH
	P. Dudley	LVR NEI
	S. Paul, Chief, Unit on Preclinical Pharm.	CPB NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH

COOPERATING UNITS (if any)
Laboratory of Vision Research, NEI
Laboratory of Neurophysiology, DBBR, NIMH
Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to develop a neuropharmacological approach to the mechanisms regulating circadian rhythms and the effects of light. Rhythms of indoleamine metabolism in the rat and chick pineal, activity rhythms in rat and hamster, and aspects of biochemistry in the rat and bovine retina have been examined.

The effects of light on the circadian rhythms in rat pineal can be mimicked by injections of carbachol which appears to act on a nicotinic receptor. Carbachol also affects the activity rhythms of rats and hamsters. Diazepam receptors are present in the rat retina. Light and darkness modulate phospholipid metabolism in the retina.

Proposed Course of Project: The mechanisms generating and regulating circadian rhythms and the effects of light will be explored further.

Publications:

Zatz, M. and Axelrod, J.: Regulation of sensitivity to ß-adrenergic stimulation in the rat pineal. In Karlin, A., Tennyson, V.M. and Vogel, H.J. (Eds.): Neuronal Information Transfer. New York, Academic Press, 1978, pp. 47-58.

O'Dea, R.F., Gagnon, C. and Zatz, M.: Regulation of cyclic GMP in the rat pineal and posterior pituitary glands. J. Neurochem., 31: 733-738, 1978.

Zatz, M., Kebabian, J.W. and O'Dea, R.F.: Regulation of ß-adrenergic function in the rat pineal gland. In Birnbaumer, L. and O'Malley, B.W. (Eds.): Receptors and Hormone Action, Vol. III. New York, Academic Press, 1978, pp. 196-219.

Zatz, M.: Sensitivity and cyclic nucleotides in the rat pineal gland. In Nir, I., Reiter, R.J. and Wurtman, R.J. (Eds.): The Pineal Gland. New York, Springer-Verlag, 1978, pp. 97-114.

Zatz, M. and Romero, J.A.: Effects of calcium-free medium on the induction of serotonin N-acetyltransferase in the rat pineal. Biochem. Pharm., 27: 2549-2553, 1978.

Zatz, M. and Brownstein, M.J.: Central depressants rapidly reduce nocturnal serotonin-N-acetyltransferase activity in the rat pineal gland. Brain Res., 160: 381-385, 1979.

Zatz, M. and Brownstein, M.J.: Intraventricular carbachol mimics the effects of light on the circadian rhythm in the rat pineal gland. Science, 203: 358-361, 1979.

Zatz, M.: Low concentrations of lithium inhibit the synthesis of cyclic AMP and cyclic GMP in the rat pineal gland. J. Neurochem., in press, 1979.

Project Description:

Objectives: To develop a neuropharmacological approach to the mechanisms generating and regulating circadian rhythms. To explore the biochemical consequences of light and darkness exposure.

Methods Employed: Biochemical, anatomical, pharmacological, behavioral, and radioactive tracer techniques.

Major Findings: The circadian rhythms in rat pineal serotonin N-acetyltransferase activity can be modified by drugs acting on the central nervous system. The acute effects of light can be mimicked by several drugs injected into the brain. Of these only carbachol was able to cause a phase shift in the temporal pattern of enzyme activity indicating an effect on the mechanism generating circadian rhythmicity. The acute effect of carbachol appears to be mediated by a nicotinic receptor. It was not blocked by muscarinic blockers but could be blocked by tubocurarine or α-bungarotoxin. Bungarotoxin was also able to block the acute effect of light on the rat pineal.

Intraventricular carbachol also had prominent effects on behavioral activity rhythms in rat and hamster.

Chick pineals show a circadian rhythm in indoleamine metabolism similar to that of rats. However, unlike rats, their rhythm is not driven by adrenergic stimulation or increased cyclic nucleotides. The chick pineal displays endogenous "time-keeping", and perhaps rhythmicity, in explant culture. It is also directly photosensitive.

The rat retina contains diazepam receptors. Most are of the "brain" type, but some of the "kidney" type are also present. The "brain" type receptors are modulated by GABA.

Rat and bovine retina contain enzymes which methylate phosphatidyl ethanolamine to form phosphatidyl choline. These enzymes are present in purified rod outer segments. In whole retina they are more active in light than in darkness.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, and pineal as well as other physiological functions. These rhythms are profoundly affected by light-dark cycles. Information about light for these "nonvisual" functions is transduced in the retina in mammals, and at least in part, in the pineal in lower vertebrates. Elucidation of the mechanisms generating or regulating circadian rhythms, and the "nonvisual" effects of light, are of broad clinical and biological interest.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00423-06 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies on Protein Carboxymethylation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Gagnon, Guest Worker	LCS NIMH
	W. Strittmatter, Staff Fellow	LCS NIMH
	J. Axelrod, Chief, Section on Pharm.	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was completed and is cancelled.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00424-04 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biologically Active Peptides in the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Brownstein, Medical Officer (Research)	LCS NIMH
	R. Eskay Staff Fellow	LCS NIMH
Other:	H. Gainer, Chief, Functional Neurochemical Sect.	LDN NICHHD
	J. Russell	LDN NICHHD
	D. Fink	LDN NICHHD
	Y.P. Loh	LDN NICHHD
	D. Krieger, Prof. Med., Mt. Sinai School of Med., NYC	
	A. Liotta, Dept. Med., Mt. Sinai School of Med., NYC	
	J. Roth, Chief, Diabetes Branch	IRP NIAMDD
	J. Havrankova, Diabetes Branch	IRP NIAMDD
	J. Rosenzweig	IRP NIAMDD
	J. Martin, Dept. Neurology, Mass.Gen. Hospital, Boston	
	O. Borstad, Dept. Neurology, Mass.Gen. Hospital, Boston	
	J. Kebabian	ET NINCDS

COOPERATING UNITS (if any)

Harvard Medical School; Mt. Sinai School of Medicine; Mass. General Hospital

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued to study the distribution of peptide-containing cells in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate peptide secretion. Our studies of a number of peptides have contributed to a better understanding of the cell biology of peptidergic neurons and of their role in the brain.

PROJECT DESCRIPTION:

Objectives: To locate specific groups of neurons in the central nervous system which synthesize and secrete peptides; to determine the biochemical mechanisms underlying the synthesis of peptides by neurosecretory cells; to study interactions between peptidergic and non-peptidergic neurons.

Methods Employed: Biosynthetic, enzymatic and histologic procedures; microdissection techniques; immunocytochemistry.

Major Findings: We have shown that neurons of the hypothalamo-neurohypophyseal system manufacture two 20,000 MW neurophysin precursors. One of these is not made by Brattleboro rats (animals with hereditary diabetes insipidus) and is presumed to be the precursor of the vasopressin-associated neurophysin. This precursor has now been shown to contain both vasopressin and its neurophysin; similarly, the second precursor mentioned above is a common precursor for oxytocin and its neurophysin. The vasopressin-neurophysin precursor (pro-pressophysin) is a glycoprotein; the oxytocin-neurophysin precursor (pro-oxyphysin) is not. The reason for this difference is unknown but is under investigation.

Dr. Brownstein's and Dr. Roth's group have shown insulin is present in the brain of the rat. Brain insulin levels do not parallel peripheral levels, and up- or down-regulation of peripheral insulin receptors is not accompanied by changes in central receptors. Insulin is also found in (and manufactured by) a number of extrapancreatic tissues besides brain. Thus, insulin may have an important and rather general role inside of cells.

The distribution of α-MSH in the rat brain has been determined by Dr. Eskay and Dr. Brownstein. Several limbic structures have especially large amounts of this peptide. The primary source of the α-MSH in the brain was shown to be neurons in the arcuate nucleus. Monosodium glutamate, a commonly used food additive, is known to be toxic to arcuate neurons; administration of this salt to neonatal animals resulted in the loss of 90% of the α-MSH from the central nervous system.

Chemical characterization of the melanotropic peptides in the brain is under way. It is evident that there are melanotropic substances in addition to α-MSH in the nervous system.

The release of α-MSH from dispersed cells of the intermediate lobe of the pituitary has been examined. This *in vitro* system has already proven to be very useful for studying the pharmacology of noradrenaline and dopamine. Isoproterenol (ℓ-) enhances the release of α-MSH, whereas dopamine or dopamine agonists inhibit its release. The isoproterenol-stimulated release of α-MSH seems to be mediated by an increase in intracellular c-AMP; the action of dopamine is independent of changes in c-AMP levels.

Somatostatin, the growth hormone releasing inhibiting hormone, is found in the hypothalamus, in extrahypothalamic brain regions, and in the periphery.

We have now shown that this peptide is present in the retina as well. By using a variety of techniques we hope to determine which cells make this and other active agents in the retina.

Significance to Biomedical Research: Peptides secreted by neurons in the CNS appear to act not only as factors which control the anterior pituitary but also as agents (neurotransmitters) which allow nerve cells to interact with one another. Thus it is important to develop methods for studying these compounds.

Proposed Course of Project: The effect of endocrinological surgical and pharmacological manipulations on peptide secretion will be studied.

PUBLICATIONS:

Havrankova, J., Schmechel, D., Roth, J., and Brownstein, M.J.: Identification of insulin in rat brain. Proc. Natl. Acad. Sci. USA 75: 5737-5741, 1978.

Palkovits, M., Mroz, E.A., Brownstein, M.J., and Leeman, S.E.: Descending substance P-containing pathway: A component of the ansa lenticularis. Brain Res. 156: 124-128, 1978.

Roizen, M.F., White, P.F., Eger, II, E.I., and Brownstein, M.J.: Effects of ablation of serotonin or norepinephrine brain-stem areas on halothane and cyclopropane MACs in rats. Anesthesiology 49: 252-255, 1978.

Liotta, A., Gildersleeve, D., Brownstein, M.J., and Krieger, D.T.: Biosynthesis in vitro of immunoreactive 31,000-dalton corticotropin/β-endorphin-like material by bovine hypothalamus. Proc. Natl. Acad. Sci. USA 76: 1448-1452, 1979.

Zatz, M. and Brownstein, M.J.: Central depressants rapidly reduce nocturnal serotonin N-acetyltransferase activity in the rat pineal gland. Brain Res. 160: 381-385, 1979.

Zatz, M. and Brownstein, M.J.: Intraventricular carbachol mimics the effects of light on the circadian rhythm in the rat pineal gland. Science 203: 358-361, 1979.

Nilaver, G., Zimmerman, E.A., Defendini, R., Liotta, A.S., Krieger, D.T., and Brownstein, M.J.: Adrenocorticotropin and β-lipotropin in the hypothalamus. J. Cell Biol. 81: 50-58, 1979.

Gainer, H. and Brownstein, M.J.: Identification of the Precursors of the Rat Neurophysins. In Vincent, J.-D. and Kordon, C. (Eds.): Biologie Cellulaire des Processus Neurosecretoires Hypothalamiques. Bordeaux, Centre National de la Recherche Scientifique, 1977, pp. 525-541.

Brownstein, M.J.: Serotonin, Histamine and the Purines. In Siegel, G.S. _et al_. (Eds.): _Basic Neurochemistry_. Little, Brown & Co. (in press).

Gainer, H. and Brownstein, M.J.: Neuropeptides. In _Basic Neurochemistry_ (in press).

Russell, J., Brownstein, M.J., and Gainer, H.: Biosynthesis of the Common Precursors of Vasopressin, Oxytocin, and their Respective Neurophysins. In Barker, J. and Smith, T. (Eds.): _The Role of Peptides in Neuronal Function_. Marcel Dekker & Co. (in press).

Eskay, R., Brownstein, M.J., and Long, R.: Reduction of α-MSH levels in the adult rat brain following monosodium glutamate treatment of neonatal animals. _Science_ (in press).

Brownstein, M.J.: The Distribution of Hypothalamic Hormone. In Motta, M. (Ed.): _Endocrine Functions of Brain_. Raven Press (in press).

Havrankova, J., Brownstein, M.J., and Roth, J.: Concentrations of insulin and of insulin receptors in the brain are independent of peripheral insulin levels. _J. Clin. Invest_. (in press).

Krieger, D.T., Liotta, A., Hauser, H., and Brownstein, M.J.: Effect of stress, ACTH, or cortico steroid treatment, adrenalectomy, or hypophysectomy on hypothalamic immunoreactive ACTH concentrations. _Endocrinology_ (in press).

Rorstad, O., Brownstein, M.J., and Martin, J.: Demonstration of immunoreactive and biologically active somatostatin-like material in the rat retina. _Proc. Natl. Acad. Sci. USA_ (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00425-03 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Peripheral and Central Catecholamines in Hypertension and Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Saavedra, Visiting Scientist	LCS NIMH
Others:	R. Kvetnansky, Visiting Scientist	LCS NIMH
	P. Guicheney, Guest Worker	LCS NIMH
	R. Del Carmine, Guest Worker	LCS NIMH
	W. Oertel, Guest Worker	LCS NIMH
	M. Torda, Visiting Fellow	LCS NIMH
	J. Kebabian, Research Associate	ETB NINCDS
	D. Murphy, Chief, Clinical Neuropharm. Br.	CPB NIMH
	A. Negro Vilar, Assoc. Prof. Southwestern Univ.	
	P. Setler, Pharmacologist, Smith, Kline & French	
	N. Alexander, Assoc. Prof., Univ. Southern California	
	E. Krieger, Professor, Univ. Sao Paulo, Brazil	

COOPERATING UNITS (if any) Experimental Therapeutics Branch, NINCDS
Clinical Neuropharmacology Branch, NIMH; Smith, Kline & French Labs.
Southwestern University; University of Southern California, Univ. of Sao Paulo, Brazil

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of catecholamines in the central and peripheral nervous system in experimental and genetic hypertension and stress. Changes in brain adrenaline and noradrenaline occur in all forms of hypertension studied (DOCA-salt, neurogenic, spontaneous and sodium dependent). Increased synthesis of adrenomedullary catecholamines is present in all types of hypertension. Acute and chronic stress also result in similar changes in brain adrenaline metabolism.

Sodium dependent hypertensive rats (Dahl rats) show specific changes in brain catecholamines. Some of these changes are related to the hypertension and some to the ingestion of a high sodium diet.

Genetic spontaneously hypertensive rats present changes in vasopressin and somatostatin levels in brain under normal conditions and after acute stress. Rats with diabetes insipidus (Brattleboro rats) show localized and specific changes in brain catecholamine metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of peripheral and central catecholamines in the nervous system in experimental and genetic hypertension and stress and to study the interaction between catecholamines and other putative neurotransmitters in these and other neuroendocrine states.

Methods Employed: Microdissection of brain nuclei; selective lesions on brain nuclei by specific neurotoxins; enzymatic isotopic micromethods for the measurement of biogenic amines and related enzymes. Radioimmunoassays for neuropeptides; immunofluorescent techniques to localize brain peptides; surgical baroreceptor deafferentation.

Major Findings: Neurogenically hypertensive (sino-aortic denervated) rats show increased activity of tyrosine hydroxylase and increased dopamine levels in the adrenal medulla three days after operation. These results suggest an early activation of peripheral catecholamine synthesis.

These rats also show changes in catecholamine levels in the anterior hypothalamic nuclei and in the area A_1 of the brain stem, and in circumventricular organs.

Sodium dependent, hypertensive rats (Dahl rats) and their normotensive controls have been examined under low and high sodium diet. Plasma, adrenomedullary and brain catecholamines have been analyzed. Under a high sodium diet, sensitive, hypertensive rats increase their synthesis of adrenomedullary catecholamines, whereas resistant, normotensive animals actually decrease such synthesis. Several different patterns of response occur in brain catecholamine neurons of sensitive and resistant rats after a high sodium load.

A localization of specific groups of brain noradrenergic and adrenergic neurons, related to the regulation of fluid and salt intake, is now possible. The role of such neurons in the development of sodium-sensitive hypertension can be further clarified.

Rats homozygous for diabetes insipidus (Brattleboro rats) show specific and localized changes in catecholamine metabolism in selected hypothalamic nuclei. These changes include decreases in dopamine, norepinephrine and epinephrine concentrations in areas related to vasopressin production.

Spontaneously genetic hypertensive rats show differences in the vasopressin content of specific brain nuclei when compared with normotensive controls. After acute stress, a totally different change in vasopressin and somatostatin levels occurs in these two groups of animals. These results support the hypothesis of the involvement of brain neuropeptides in the central control of blood pressure and in the central response to stress.

Phenylethanolamine N-methyltransferase (PNMT) activity is increased in selective brain stem areas of rats subjected to acute and chronic immobilization stress. In acute stress there is a concommitant decrease in epinephrine levels in brain stem.

Epinephrine has been shown to be present in the rat pineal gland at relatively low concentrations. Neurogenically hypertensive and genetically hypertensive rats have more pineal epinephrine than their normotensive controls.

Spontaneously hypertensive rats have increased number and increased affinity of ß-adrenergic receptors than their normotensive controls, Wistar Kyoto, and than the normotensive Sprague-Dawley rats. The increased number of receptors seems to be localized to atrial and nodal structures, and the increased affinity of receptors to the heart ventricles.

Normal endogenous levels of octopamine can be measured in human blood platelets collected from as little as 2 ml of blood. The octopamine levels increase in patients and controls after administration of monoamine oxidase inhibitors. Changes in platelet octopamine can reflect the degree of in vivo inhibition of monoamine oxidase.

Proposed Course of Project: Other neurotransmitters or neuromodulators, like enkephalins, endorphins, serotonin, histamine, GABA and acetylcholine, will be studied in selective brain areas of the neuroendocrine and hypertensive models available. Special interest will be focused on the interaction between those neuromodulators and catecholamines. A combination of drug treatments, endocrine ablations, central lesions and other neuroendocrine manipulations will be performed in these hypertension and neuroendocrine models.

New micromethods for the study of catecholamine metabolism will be developed. Dopa, 5-hydroxytryptophan, normetanephrine and methoxytyramine could then be measured at the picogram level. These and other microenzymatic techniques will be adopted for the study of neurotransmitters in human cerebrospinal fluid.

Significance to Biomedical Research: The determination of endogenous levels of octopamine in human platelets isolated from as little as 2 ml of blood can be used as an index of in vivo inhibition of this MAO during drug therapy in depressive patients. The confirmation of a role of central and peripheral catecholamines, noradrenaline and adrenaline, in the development and maintenance of several types of hypertension, and the efficacy of inhibitors of adrenaline synthesis to decrease high blood pressure could result in the development of new drugs for the treatment of hypertension.

Publications:

Saavedra, J.M., Setler, P.E. and Kebabian, J.W.: Biochemical changes accompanying unilateral 6-hydroxydopamine in the rat substantia nigra. Brain Res., 151, 339-352, 1978.

Kvetnansky, R., Kopin, I.J. and Saavedra, J.M.: Changes in epinephrine in individual hypothalamic nuclei after immobilization stress. Brain Res., 155: 387-390, 1978.

Saavedra, J.M. and Grobecker, H.: Central and peripheral catecholamines in experimental and genetic hypertension. In Meyer, P. and Schmitt, H. (Eds.): Nervous System and Hypertension. Paris and New York, Wiley-Flammarion, 1979, pp. 223-233.

Saavedra, J.M., Kvetnansky, R. and Kopin, I.J.: Adrenaline, noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. Brain Res., 160: 271-280, 1979.

Saavedra, J.M.: Adrenaline levels in brain stem nuclei and effects of a PNMT inhibitor on spontaneously hypertensive rats. Brain Res., 166: 283-292, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00426-03 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Catecholestrogens: Synthesis and Metabolism after Endocrine Manipulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hoffman, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Other:	S. Paul, Chief, Unit on Preclinical Pharm.	CPB NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, DCBR NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Catecholestrogens are synthesized by 2-hydroxylation in liver, brain and other organs and by demethylation in liver alone. The cytochrome P450 enzyme system is involved in all of these reactions. Breast tumors, but not normal breast tissue, can also synthesize and metabolize the catecholestrogens. In brain and liver catecholestrogen synthesis is diminished in chronically hypothyroid rats and in castrated male rats. Phenobarbitol increases the synthesis in liver by demethylation only.

Other cytochrome P450 dependent reactions occur in brain. P-hydroxyamphetamine, tyramine and octopamine are all converted to catecholamines, but phenolic narcotics are not metabolized to their catechol derivations in brain.

Project Description:

Objectives: To utilize sensitive radioenzymatic methods to study the regulation of estrogen and catecholestrogen metabolism in endocrine disease and to study the neuropharmacology of CNS cytochrome P450 drug transformations.

Methods Used: Enzymatic, endocrinologic, pharmacologic, tissue culture.

Major Findings: The metabolism of catecholestrogens has been under study. In experiments designed to characterize the enzyme that synthesizes catecholestrogen from estrogen it was shown that the enzyme is not inducible by phenobarbital or 3-methylcholanthrene. Brain and liver enzyme activities fall after castration or prolonged hypothyroidism. Nearly all rat tissues studied have catecholestrogen synthesizing activity, but male tissues have higher specific activity than female. Human breast tumors, but not normal breast tissue, also have catecholestrogen-forming activity and our early studies indicate that catecholestrogens stimulate cultured human breast tumor cells in culture.

Catecholestrogen synthesis can also occur in liver via enzymatic demethylation of 2 methoxyestrogens. A rapid, sensitive and specific assay for this demethylase was devised. The enzyme(s) responsible for this reaction is a cytochrome P450 which can be induced by phenobarbital but not by 3-methylcolanthrene. No detectable demethylation occurs in brain, however.

The cytochrome P450 enzyme system in brain may play a significant role in the local control of CNS drug concentrations. It was shown that p-hydroxyamphetamine is converted to a catecholamine by this brain hydroxylase. Phenolic narcotics, however, do not appear to be converted to their catechol metabolites in brain. Naturally occurring amines, tyramine and octopamine, are substrates for this enzyme. Their hydroxylation represents an alternative pathway for catecholamine synthesis in brain.

Significance to Biomedical Research: Catecholestrogens are important metabolites of endogenous and exogenous estrogens. The presence of a catecholestrogen synthesizing capacity in breast neoplasms may provide a clue to the regulation of mammary cancer. The altered synthesis of catecholestrogens in hypo- and hyperthyroidism may provide a link between the known association between thyroid disease and breast cancer.

Proposed Course of Project: Studies on the tissue distribution of the demethylating enzyme will continue. Tests will be performed on the physiological effects of the catecholestrogens in a pituitary tissue culture cell line, looking specifically for interactions between catecholestrogens and cell membranes.

The use of COMT, present in high concentrations in breast tumors, as a serum marker for metastatic breast cancer will also be investigated.

Publications:

Hoffman, A.R., Sastry, B.V.R. and Axelrod, J.: The formation of α-methyldopamine ("catecholamphetamine") from p-hydroxyamphetamine by rat brain microsomes. Pharmacology (in press), 1979.

Hoffman, A.R., Paul, S.M. and Axelrod, J.: Catecholestrogen synthesis and metabolism by human breast tumors *in vitro*. Cancer Research (in press), 1979.

Hoffman, A.R., Paul, S.M. and Axelrod, J.: Estrogen-2-hydroxylase in the rat: Distribution and response to hormonal manipulation. *Biochem. Pharmacol.* (in press), 1979.

Paul, S.M., Hoffman, A.R. and Axelrod, J.: Catecholestrogens: Synthesis and metabolism in brain and other endocrine tissues. In Ganong, W. and Martini, L. (Eds.): *Frontiers of Neuroendocrinology, Vol. 6*. (in press), 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00427-02 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Phospholipid Methylation and Membrane Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Hirata, Visiting Scientist	LCS NIMH
	W. Strittmatter, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
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Other:	J. Tallman, Research Biologist	BPB NIMH
	E. Schiffmann, Research Chemist	LBDA NIDR

COOPERATING UNITS (if any)
Behavioral Psychiatry Branch, DCBR, IRP, NIMH
Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

ß-adrenergic agonists stimulate phospholipid methylation in Hela cells and astrocytomas. Benzodiazapam acid anti-anxiety drugs also stimulate phospholipid methylation in astrocytoma cells. Ca^{2+} ATPase activity is increased after synthesis of phosphatidylcholine is elevated. An association between histamine release in mast cells and phosphatidylserine decarboxylation and phospholipid methylation was observed. Also a close relationship between lymphocyte mitogenesis, phospholipid methylation and arachidonic liberation was found. Chemotactic peptides caused an increased turnover of methylated phospholipids and release of arachidonic acid. Two phospholipid methylating enzymes were found in brain nerve endings.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of phospholipid methylation in the function of mast cells, lymphocytes, leukocytes and brain.

Methods Employed: Enzymatic and radiometric.

Major Findings: Work done during the past year has shown that phospholipid methylation influences many membrane events. We have previously found that the stimulation of ß-adrenergic receptors in rat reticulocytes increases phospholipid methylation, membrane fluidity, and coupling of the ß-adrenergic receptor and adenylate cyclase. ß-adrenergic agonists stimulate methylation in Hela cells and astrocytomas. Benzodiazapam, an anti-anxiety drug, also stimulates phospholipid methylation in astrocytoma cells. Experiments indicate that phospholipid methylation can occur on separate domains on the membrane. Rapid desensitization of ß-adrenergic receptors is prevented by inhibiting phospholipase A_2, an enzyme that metabolizes methylated phospholipids. Ca^{2+}ATPase activity is elevated after methylation of membrane phospholipids is increased.

Lectins such as concanavalin A (Con A) interacts with cell surface receptors and releases histamine from mast cells. The lectin also stimulates phospholipid methylation and degradation of methylated phospholipids. A close association between phospholipid methylation and histamine release was found. Con A also stimulates the conversion of phosphatidylserine to methylated phospholipids. Mitogenesis in rat lymphocytes has been found to be associated with phospholipid methylation. A close correlation between Con A-evoked mitogenesis, phospholipid methylation and arachidonic acid liberation was observed. Peptides that cause chemotaxis in rabbit leukocytes increase turnover of methylated phospholipids. The rapid turnover of methylated phospholipids by chemoattractant peptides leads to an increased release of arachidonic acid, the precursor of prostaglandins.

Two enzymes have been found in brain that convert phosphatidyl ethanolamine to phosphatidyl choline. These enzymes are highly localized in nerve terminals.

Significance to Biomedical Research: Phospholipid methylation appears to be involved in histamine release (allergy), lymphocyte mitogenesis (antibody formation), leukocyte chemotaxis (inflammation and phagocytosis).

Proposed Course of Project: Phospholipid methyltransferases will be purified and characterized, and antibodies to these enzymes will be made. Clustering of methyltransferases in various receptors in membranes will be examined. The role of phospholipid methylation in acetylcholine synthesis in brain will be studied.

ublications

Gagnon, C., Axelrod, J. and Brownstein, M.J.: Protein carboxymethylation: Effects of 2% sodium chloride administration on protein carboxymethylase and its endogenous substrates in rat posterior pituitary. Life Sci., 22: 2155-2164, 1978.

Hirata, F. and Axelrod, J.: Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity. Nature, 275: 219-220, 1978.

Strittmatter, W.J., Gagnon, C. and Axelrod, J.: Beta-adrenergic stimulation of protein carboxymethylation and amylase secretion in rat parotid gland. J. Pharmacol. Exp. Ther., 207: 419-424, 1978.

Axelrod, J.: Regulation of the beta-adrenergic receptor in the pineal gland and red cell membranes. In Fuxe, K., Hokfelt, T. and Luft, R. (Eds.): Central Regulation of the Endocrine System. New York, Plenum Publishing Co., 1979, pp. 149-156.

Gagnon, C. and Axelrod, J.: Subcellular localization of protein carboxylmethylase and its substrates in rat pituitary lobes. J. Neurochem., 32: 567-572, 1979.

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Hirata, F., Strittmatter, W.J. and Axelrod, J.: Beta-adrenergic receptor agonists increase phospholipid methylation, membrane fluidity and beta-adrenergic receptor-adenylate cyclase coupling. Proc. Natl. Acad. Sci., USA, 76: 368-372, 1979.

Strittmatter, W.J., Hirata, F. and Axelrod, J.: Increased Ca^{2+}-activity associated with methylation of phospholipids in human erythrocytes. Biochem. Biophys. Res. Commun., 88: 147-153, 1979.

Strittmatter, W.J., Hirata, F. and Axelrod, J.: Phospholipid methylation unmasks cryptic beta-adrenergic receptors in rat reticulocytes. Science, 204: 1207-1209, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00428-02 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Benzodiazepine Receptors in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Paul, Staff Psychiatrist	CPB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is being continued by PI in his present assignment in the Clinical Psychobiology Branch, DCBR NIMH.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00401-14 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Formation, Release, Disposition, and Metabolism of Neurotransmitters in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	David C. Jimerson	Senior Staff Fellow	LCS NIMH
	Richard McCarty	Guest Worker	LCS NIMH
	Richard Kvetnansky	Visiting Scientist	LCS NIMH
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	Virginia K. Weise	Chemist	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Anthony P. Zavadil	Guest Worker	LCS NIMH
	Marian S. Kafka	Psychologist	BP NIMH

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Section on Histopharmacology

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.8	1.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to elucidate the biochemical mechanisms controlling the synthesis, storage, release, action and termination of action of neurotransmitters and to determine how genetic factors, environmental stimuli, or drugs influence these control mechanisms. The autonomic responses to stress appear to be genetically determined and differ among strains of rats. There is a relationship between the degree of behavioral activation and elevation of plasma catecholamines found during stress. In addition to differences in the magnitude of response of the central nervous system, in spontaneously hypertensive rats there is a greater sensitivity of the arterioles to norepinephrine, whether released from the nerve endings or administered intravenously. The central control of sympathadrenal activity involves cholinergic mechanisms which interact with dopaminergic neurones as well. Presynaptic α_2-adrenoceptors are present on axonal sprouts. The affinity of various agonists to these receptors parallels their ability to inhibit release of ^{3}H-NE.

PROJECT DESCRIPTION:

Catecholamines in plasma are derived from the adrenal medulla (which secretes mainly epinephrine) and sympathetic nerve endings (which release norepinephrine). Studies in pithed rats have established that the rate of entry of norepinephrine into plasma is directly proportional to the rate of sympathetic nerve impulse flow, but that the blood pressure increases are proportional to the logarithm of the amount of norepinephrine released. Catecholamines derived from the adrenal medulla have only slight and transient effects on blood pressure, but may be important for metabolic responses.

Techniques for measurement of plasma levels of catecholamine metabolites have been developed in collaboration with the Unit on Mass Spectroscopy. These techniques provide another dimension to the methods developed in this laboratory for measurement of plasma levels of catecholamines and for obtaining blood samples from intact, unanesthetized and freely moving rats. The plasma levels of 3, 4,dehydroxyphenyl glycol increase when catecholamines are released and the decline while plasma levels of 3-methoxy, 4-hydroxy phenyl glycol and its sulfate conjugate increase more slowly but remain elevated. Drugs (e.g., imipramine) which interfere with reuptake of norepinephrine into sympathetic nerve endings decrease the rate of appearance of the metabolites in plasma and increase the rate of entry of norepinephrine into blood. Levels of plasma EPI and NE are increased by even minor changes in the environment. Lifting the top of the cage or moving the cage to another room results in increased plasma levels of EPI and NE. The levels of these amines are strikingly elevated when animals are forcibly immobilized. Plasma levels of EPI reach peak levels (about 40-fold greater than when undisturbed) after about 20 minutes of stress and then decline to about 12-fold greater than basal. Plasma levels of NE are increased by about 6-fold throughout the interval of immobilization. There are also differences among strains in the levels of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) activity and of catecholamine content of the adrenal medulla. The least reactive strain had the highest levels of DBH and NE consistent with diminished release of DBH and slower conversion of NE and EPI (although levels of PNMT are not decreased).

There are striking differences among various genetic strains of rats in the plasma catecholamine response evoked by exposure to stress. In a study in which behavioral and plasma catecholamine responses to foot-shock were compared, it was found that there was an excellent correlation between these two parameters in seven different strains of rats. The greatest behavioral activation and the largest increments in plasma catecholamines were found in spontaneously hypertensive (SHR) rats. The normotensive Wistar Kyoto (WKY) strain from which these rats were bred has lower behavioral activation and lesser increases in plasma catecholamines, but these were still high when compared with those found in several other normotensive strains of rats. The strain with the lowest behavioral activation score also had the smallest increment in plasma catecholamines.

The hyper-responsivity to foot-shock of the plasma catecholamine response of SHR rats compared to WKY rats is present at six weeks of age, before development of hypertension, so that the hyper-responsivity is independent of increases in blood pressure (B.P.), but may contribute to its development and maintenance. The B.P. and heart rates (H.R.) could be monitored via the indwelling arterial cannulae and it was found that while transfer of the animals to the shock cage caused greater increases in B.P. and H.R. in SHR than WKY rats, during the shock intervals the B.P. of SHR decreased. Since H.R.'s were increased, the fall in B.P. was presumably due to vasodilation attending the muscular effort and as a result of the released epinephrine.

A similar pattern emerges when forced immobilization is used as the stressor. During 2 hours of immobilization, circulating levels of NE, EPI, and corticosterone were higher, and remained higher for several hours, in SHR than in WKY rats, but with repeated immobilization there was a reduction in evoked adrenalmedullary secretion and increase in B.P. of SHR rats. One day after the last of a series of six daily intervals of forced immobilization, basal levels of plasma catecholamines were significantly higher, but B.P. was significantly lowered in SHR but not WKY rats. These results suggest that the muscular effort or repeated exposure to high catecholamine levels resulted in reduction of peripheral resistance in SHR rats.

Several factors appear to contribute to the differences in B.P. response to stress in SHR and WKY rats. There is a greater central activation of the nervous system in SHR rats with more intense stimulation of the sympathetic outflow. Studies in pithed SHR and WKY rats show that there is no difference in the amounts of norepinephrine released when the same intensity of stimulation is applied to the sympathetic outflow, but in SHR rats, the B.P. response is greater. These observations suggested that the sensitivity of the blood pressure to norephinephrine released at sympathetic nerve endings was greater in SHR rats. This conclusion was supported by the observation that the B.P. elevation induced by administration of catecholamines was greater in SHR rats than in WKY rats. There appear to be two factors responsible for this difference. In young SHR rats the β-adrenoceptor mediated vasodilatory responses to catecholamines are less than in WKY rats so there is a net enhancement of arterial resistance to flow. In older SHR rats, however, structural changes appear to account for the greater arterial resistance.

Oxotremorine, a cholinergic (muscarinic-type) agonist, induces tremor in rats by an action in the central nervous system. It has been found to also cause a centrally mediated increase in plasma catecholamines. Since the intensity of the tremor is reduced by removing the adrenal medulla, by treatment with 6-hydroxydopamine (which produces chemical sympathectomy) or by administration of a β-adrenergic blocking agent which does not prevent the oxotremorine-induced increases in plasma catecholamines), it appears that release of peripheral catecholamines augments the centrally-induced tremor by an action on peripheral β_2-receptors. This may be related to the well known occurence of tremor attending fear responses in humans.

In cultered rat sympathetic ganglia, regenerating axonal sprouts have the properties of sympathetic nerve endings. The axonal sprouts take up ^{3}H-norepinephrine which is released when the neurone is depolarized. They contain α-adrenoceptors stimulation of which inhibit release of ^{3}H-norepinephrine. The order of potency of α-adrenoceptor agonists in inhibiting release parallels their potency in displacing ^{3}H-dihydroergotamine from the membranes obtained from the cultured ganglia; this is characteristic of the α_2-adrenoceptors.

PUBLICATIONS:

Chiueh, C.C. and Kopin, I.J.: Hyperadrenergic responsivity of hypertensive SHR rat to indirect measurement of blood pressure. Am. J. Physiol., 234: H690-H695, 1978.

Kopin, I.J.: Biochemical assessment of peripheral adrenergic activity. In Paton, D.M. (Ed.): The Release of Catecholamines from Adrenergic Neurons. Pergamon Press, New York, 1979 p. 355-372.

Kvetnansky, R., Sun, C.L., Lake, C.R., Thoa, N., Torda, T. and Kopin, I.J.: Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine and dopamine-β-hydroxylase. Endocrinology, 103: 1868-1874, 1978.

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Kvetnansky, R., McCarty, R., Thoa, N.B. Lake, R. and Kopin, I.J.: Sympathoadrenal responses of spontaneously hypertensive rats to immobilization stress. Am. J. Physiol., 236: H457-H462, 1979.

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Weinstock, M., Zavadil, III, A.P., Chiueh, C.C. and Kopin, I.J.: The effect of oxotremorine on blood pressure and plasma catecholamines in conscious and anesthetized rats. Life Sci., 24: 301-310, 1979.

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Kvetnansky, R., Weise, V.K., Thoa, N.B. and Kopin, I.J.: Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats. J. Pharmacol. Exp. Ther., 209: 287-291, 1979.

Yamaguchi, I. and Kopin, I.J.: Direct Stimulation of Sympathetic outflow and plasma levels of norepinephrine and epinephrine of pithed rats. In: E. Usdin, I. Kopin, and J. Barchas (Eds.): Catecholamines: Basic and Clinical Frontiers, Pergamon Press, New York, 1979, pp 924-926.

Jimerson, D.C., Sun, C.L., Yamaguchi, I. and Kopin, I.J.: Plasma levels of glycol metabolites and presynaptic metabolism of norepinephrine (NE). In: E. Usdin, I. Kopin and J. Barchas (Eds.): Catecholamines: Basic and Clinical Frontiers, Pergamon Press, New York, 1979, pp 927-929.

Kvetnansky, R., Kopin, I.J., and Klein, D.C.: Stress increases pineal epinephrine.: Commun. Psychopharmacol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00402-07 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

CNS Regulation of Autonomic and Endocrine Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Joseph DiMicco	Staff Fellow	LCS NIMH
	Gertrude Maengwyn-Davies	Guest Worker	LCS NIMH
	Ching-Long Sun	Visiting Fellow	LCS NIMH
	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Marta Weinstock	Visiting Scientist	LCS NIMH
	Anthony P. Zavadil	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)
Section on Histopharmacology
Section on Pharmacology

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the regions of brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic and autonomic responses to internal stimuli (e.g., blood glucose, O_2-tension, hormonal state, body temperature, etc.) and to various types of stress (immobilization, foot-shock, hemorrhage, etc.), and the effects of drugs on these systems.

PROJECT DESCRIPTION:

Turnover rates of neurotransmitters and alterations in the levels of critical enzymes which control the rate of synthesis of these neurotransmitters reflect the degree of activity of specific neurones. Using techniques for microdisection and microassay of these enzymes, neurotransmitters or their metabolites, the effects of severing tracts, destroying nuclei, exposure to stress or administration of drugs have been examined. The effects of these lesions or procedures on physiological responses have also been examined to determine the role of neurotransmitter systems in specific areas in modulating these responses.

The turnover rate of NE in the hypothalamus of spontaneously hypertensive (SHR) rats is greater than in normotensive (WKY) rats of the same strain, but turnover of this CA was not increased in the brain stem. This difference was greater in older SHR animals after hypertension is established.

In aggressive strains of mice there appear to be higher levels and increased turnover rates of NE in the hypothalamus, frontal cortex and caudate nucleus than in less aggressive strains. Other areas of brain have higher NE levels but turnover rates are not significantly different.

Epinephrine and phenylethanolamine-N-methyltransferase (PHMT), the epinephrine-forming enzyme, are found in the same small regions of brain. The results suggest that there is an epinephrine-containing neuronal system with cell bodies in the brain stem. The axons from these cells innervate other areas of the brain stem, the locus coeruleus and the hypothalamus. After an interval of forced immobilization of rats, epinephrine levels in the periventricular, ventromedial, and arcuate nuclei are decreased, while in the median eminence, levels of epinephrine are increased, suggesting that the epinephrine-containing cells play a role in the response to stress.

Oxotremorine is a muscarinic cholinergic drug which produces tremor in rats. The drug induces increases in dopamine (DA) turnover and thereby elevates levels of homovanillic acid (HVA) in the Corpus Striatum (C.S.) and N. Accumbens (N.A.). Both propranolol (a β-receptor blocking agent) and clonidine (an α-receptor stimulator) diminish the oxotremorine-induced increases in HVA in the C.S. but not in the N.A. Haloperidol, which blocks DA receptors also increases levels of HVA in the C.S. and N.A., but its effects are not blocked by either propranolol or clonidine. These results indicated that norepinephrine may mediate the cholinergic stimulation of release of DA in the C.S. but not in the N.A.

Denervation of the baroreceptors, which is known to cause hypertension in animals, results in increased neuronal activity, reflected by enhanced uptake of ^{3}H-2-deoxyglucose, in specific nuclei of the cat brain stem. The amygdala, nucleus tractus solitarius, and several other small areas appear to have increased activity and to activate the sympathetic nervous system.

Using plasma levels of catecholamines to reflect sympathoadrenal medullary secretion and corticosterone levels to reflect release of ACTH from the pituitary, the effects of complete and partial hypothalamic deafferentation on stress-evoked endocrine responses have been examined. Complete hypothalamic deafferentation diminishes markedly the increases in catecholamines and corticosterone found during immobilization or after administration of 2-deoxyglucose (2-DG). Partial deafferentation by severing lateral hypothalamic pathways diminishes the adrenal cortical responses while anterior deafferentation enhances slightly the corticosterone release during immobilization but not after 2-DG. Posterior hypothalamic cuts prevent the elevation of catecholamines by immobilization but do not appear to affect the response to 2-DG. These results suggest that stimulation of the hypothalamus by both immobilization and 2-DG is initiated by neurones outside the hypothalamus and that both anterior and lateral pathways are involved. The discharge of catecholamines appears to involve pathways which pass through the hypothalamus and emerge posteriorly.

Direct stimulation of the hypothalamus evokes release of catecholamines (mainly epinephrine) into the plasma. Direct injection of picrotoxin into the posterior hypothalamus also elicits catecholamine release and this response is prevented by diazepam. The effects of other drugs which alter neurotransmitter release or stimulate receptors are being examined to attempt to ellucidate the neurotransmitters involved in the responses to stress. The responses to posterior hypothalamic stimulation are markedly attenuated by clonidine or α-methyldopa. These drugs are used as antihypertensive agents in patients.

PUBLICATIONS:

Kopin, I.J.: Mode of action of: Antidepressants and central stimulants. In van Praag, H.M. (Ed.): Handbook of Biological Psychiatry. New York, Marcel Dekker, Inc., (In Press).

Kvetnansky, R., Kopin, I.J. and Saavedra, J.M.: Changes in epinephrine in individual hypothalamic nuclei after immobilization stress. Brain Res., 155: 387-90, 1978.

Tizabi, Y., Thoa, N.B., Maengwyn-Davies, G.C., Kopin, I.J. and Jacobowitz, D.M.: Behavioral correlation of catecholamine concentration and turnover in discrete brain areas of three strains of mice. Brain Res., 166: 199-205, 1979.

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Sun, C.L. and Kopin, I.J.: Plasma catecholamines and direct stimulation of rat hypothalamus. In: E. Usdin, I. Kopin and J. Barchas (Eds.): Catecholamines: Basic and Clinical Frontiers, Pergamon Press, New York, 1979, pp 1422-1424.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00403-06 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Control of Catecholamines and DBH in Human Plasma and CSF

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. Raymond Lake	Staff Psychiatrist	LCS NIMH
OTHER:	Virginia K. Weise	Chemist	LCS NIMH
	Michael D. Coleman	Nephrologist, Holt Krock Med. Clin., Fort Smith, Ark.	
	Sally Anderson	Biologist	LPP NIMH
	David Lozovsky	Psychiatrist	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH
	Young Kim	Anesthesiologist	CC ANES

COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS; Holt Krock Medical Clinic, Fort Smith, Arkansas; Dept. of Medicine, Mt. Sinai Hospital, New York, N.Y.; Surgical Neurology Branch (NINCDS), NIH, Bethesda, Maryland

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine (DA), norepinephrine (NE), and epinephrine (EPI) and their metabolites and dopamine-β-hydroxylase (DBH) are present in human plasma and cerebrospinal fluid (CSF). Levels of these substances reflect the balance between their release and removal. The increases in levels of NE and EPI with standing, exertion, stress, drugs, administration, etc., are used to examine sympathetic neuronal responsivity. The responses and level of activity of the sympathetic nervous system in cardiovascular disorders (hypertension, hypotension) in alcoholism, a variety of psychiatric disorders, and after treatment with drugs are being examined. Dopamine-beta-hydroxylase (DBH) is the enzyme which converts dopamine (DA) to NE. NE and DBH can be measured in cerebrospinal fluid (CSF) and may be useful to estimate in diseases in which a defect is suspected, i.e., essential hypertension, Shy-Drager syndrome, amyotrophic lateral sclerosis (ALS), enuresis and hyperactivity in children, and schizophrenia.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Blood pressure is maintained by changes in sympathetic neuronal activity, These changes are mediated by the central nervous system which receives information from baroreceptors in the aorta and carotid sinus and volume receptors in the right atrium. Plasma levels of norepinephrine (NE) reflect acute changes in sympathetic neuronal activity because the catecholamine is rapidly metabolized and its levels are maintained by continuous secretion into the blood. Dopamine-β-hydroxylase (DBH) in plasma is derived from sympathetic nerves, but its levels are genetically determined and change slowly in any individual because it is slowly metabolized. Thus, in a single individual, changes in levels of DBH reflect chronic changes in sympathetic activity or in fluid distribution, but genetic differences account for most of the differences in DBH of plasma from different persons.

In our studies, as well as in several other reports, levels of NE in hypertensive patients are similar to those in normal subjects, but the relationship of NE levels in plasma to age found in normal subjects is not apparent in the hypertensive population, because among the hypertensive subjects there are a few young patients with high levels of plasma NE. In other studies, hypertensive patients have been found to have higher levels of plasma NE than in normotensive subjects. In these studies the relationship of plasma NE levels to age was not found. Analyses of the results of both groups of studies shows that the difference between the two groups is a result of a difference in the normal subjects. Studies in which hypertensives were found to have higher levels of plasma NE than in normal subjects had values for normal controls which were significantly lower, and had lower variance, than did the normotensive subjects in studies in which hypertensives and normotensive subjects had similar levels of plasma NE. This difference may be attributed to use of a biased group of older normal subjects. This bias may have been introduced by use of medical personnel who were familiar with hospital procedures and failed to have the usual sympathetic response to the procedures found in random normotensive and hypertensive subjects.

Decreases in plasma volume, as a result of treatment with diuretics, in normal or hypertensive patients, evoke reflex-mediated increases in sympathetic activity reflected by increased levels of plasma NE, but not DBH.

The enhanced sympathetic activity and elevation in plasma levels of NE attending treatment of hypertensive patients with drugs which block actions of catecholamines, produce vasodilation, or diminish vascular volume, can be prevented by administration of drugs, such as clonidine or α-methyl dopa, which activate α-adrenergic receptors in the central nervous system. Fenfluramine, a drug which interacts with serotonergic neurones, also diminishes plasma catecholamines by an unknown mechanism involving the central nervous system.

Resetting of the baroreceptors to "defend" the level of blood pressure at hypertensive, hypotensive levels may be a phenomenon of adaptation. In patients with cystic fibrosis blood pressure levels are maintained at a low level, perhaps because of a tendency to have low sodium levels in plasma, but there is no elevation of plasma catecholamines.

In children, imipramine and dimethylimipramine elevate blood pressure and increase plasma levels of norepinephrine, while in adults this effect is not seen. Possibly the differences between adults and children are the relatively poorly developed baroreceptor reflexes in children.

Increased levels of NE are evoked by stress, sexual excitation, hypoglycemia, and volume depletion during hemodialysis for chronic renal failure. In patients with Down's syndrome plasma NE levels are normal and respond normally to stress, but levels of DBH in plasma are low, perhaps on a genetically related basis.

Bromocriptine, clonidine, and fenfluramine decrease plasma levels of NE, while amphetamine, hydrochlorothiazide and imipramine elevate plasma NE.

The ability to assess directly noradrenergic neurotransmission in brain areas adjacent to the cerebral ventricles by assaying NE from CSF yields information about nerve function and alterations in various disease conditions or with the administration of various drugs.

We are attempting to examine possible subgroups of essential hypertension and schizophrenia for defects in central or peripheral SNS function. Preliminary data imply elevated levels of NE from CSF from patients with paranoid schizophrenia.

PUBLICATIONS:

Kopin, I.J.: Plasma catecholamines in human and experimental hypertension. In Meyer, P. and Schmitt, H. (Eds.): Proceedings of the Workshop on Nervous System and Hypertension. John Wiley & Sons (In press).

Kopin, I.J.: Central nervous system actions of anti-hypertensive drugs. Drug Therapy, (In press).

Lake, C.R., Davis, P., Ziegler, M.G., di Sant'Agnese, P.A., and Kopin, I.J.: Electrolytes and norepinephrine levels in blood of patients with Cystic Fibrosis. Clinica Chemica Acta 92: 141-146, 1979.

Lake, C.R.; Mikkelson, E.J.; Rapoport, J.L.; Zavadil III, A.P. and Kopin, I.J.: The effect of imipramine on norepinephrine and blood pressure in enuretic children. *Clin. Pharmacol. Ther*. (In press).

Lake, C.R.; Ziegler, M.G.; Coleman, M.D. and Kopin, I.J.: Sustained sympathetic hyperactivity in hydrochlorothiazide-treated hypertensives. *Clin. Pharmacol. Ther*. (In press).

Lake, C.R.; Ziegler, M.G.; Coleman, M.D. and Kopin, I.J.: Evaluation of the sympathetic nervous system in trisomy-21 (Down's syndrome). *J. Psychiatr. Res*. *15*: 1-6, 1979.

Lake, C.R.; Ziegler, M.G.; Coleman, M.D. and Kopin, I.J.: Plasma levels of norepinephrine and dopamine-β-hydroxylase in patients treated with hemodialysis for chronic renal failure. *Cardiovasc. Med*. (In press).

Kim, D.Y., Lees, D.E., Lake, C.R., Whang-Peng, J., Schuette, W., Smith, R., and Bull, J.: Acute hyperthermic potentiation of adriamycin cardiotoxicity. *JAMA* *241*: 1816-1817, 1979.

Lake, C.R.: Relationship of sympathetic nervous system tone and blood pressure. *Nephron* *23*: 84-90, 1979.

Lake, C.R., Coleman, M.D., Ziegler, M.G., and Murphy, D.L.: Fenfluramine and its effects on the sympathetic nervous system in man. *Curr. Med. Res. Opin*. *6*: 63-72, 1979.

Ziegler, M.G., Lake, C.R., Williams, A.C., Teychenne, P.F., Shoulson, I., and Steinsland, O.: Bromocriptine inhibits norepinephrine release. *Clin. Pharm. Ther*. *24*: 137-142, 1979.

Ziegler, M.G. and Lake, C.R.: Norepinephrine alterations associated with physiologic and pathologic activity of central sympathetic nervous system in man. In Wood, J.H. (Ed.): *Neurobiology of Cerebrospinal Fluid*. New York, Plenum Press, 1979, (In press)

Lake, C.R., Major, F.L., Ziegler, M.G., Ballenger, J.C., Brown, G.L., and Ebert, M.H.: The effects of disulfiram on peripheral and central norepinephrine metabolism and blood pressure. In Fann, W.E., Karacan, I., Pokorny, A.D., and Williams, R.L. (Eds.): *Phenomenology and Treatment of Alcoholism*. New York, Spectrum Publ. Co., (In press).

Roizen, M.F., Moyers, J.R., Zivin, J.A., Wilkinson, P.L., Lake, C.R., Hamilton, W.K., Chatterjee, K., and Horrigan, R.W.: Predictability of the change in plasma norepinephrine concentration in one vascular bed knowing the change in plasma norepinephrine in another vascular bed. In Usdin, E. (Ed.): *Catecholamines: Basic and Clinical Frontiers*. *Oxford, Pergamon Press*, 1979, (In press).

Wiedeking, C., Lake, C.R., and Ziegler, M.G.: Plasma noradrenaline and dopamine-β-hydroxylase during human sexual activity. *J. Psych. Res.*, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00404-08 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Growth Characteristics and Immunological Localization of Enzymes in Neurones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Donald E. Schmechel	Medical Officer	LCS NIMH
	Gad Gilad	Visiting Fellow	LCS NIMH
	Wolfgang Oertel	Guest Worker	LCS NIMH
	Virginia K. Weise	Chemist	LCS NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, NIMH and Laboratory of Neuropathology and Neuroanatomical Sciences, NIH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunological methods are being developed in attempts to define the distribution in the nervous system and other tissues of cells (neurones, glia, etc.) which contain specific enzymes associated with particular functions (e.g., production of specific neurotransmitters, hormones, etc.). The ontogeny of the specific proteins and their role in growth, differentiation and regenerative processes are being examined using these techniques.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Studies of enzyme levels during development of the rat cerebellum showed differences in the patterns of appearance of tyrosine hydroxylase, glutamine acid decarboxylase, and choline acetylase (enzymes which control synthesis of dopamine and norepinephrine, α-aminobutyric acid (GABA), and acetychaline, respectively). These enzymes reflect development of specific neuronal systems. The enzymes which control polyamine synthesis, ornithine decarboxylase and S-adenosylmethionine decarboxylase, increased transiently and declined to low levels in the adult, perhaps reflecting the role of polyamine in initiating different phases of development. After axonal injury, changes in enzyme levels in the areas of the cell bodies reflect a shift in synthesis of proteins from those concerned with structural aspects rather than those involved in neurotransmitter formation.

Antibodies to two different forms of enolase, one of which appears to be specific to neurones, whereas the other is characteristic on glia and other non-neuronal tissue. The neurone-specific enolase is present in neurones of the primitive cortex of embryos, but not in cells which are undifferentiated or have not yet begun to function as neurones. This form of the enzyme is also found in early neuroendocrine cells. These results suggest that these neurones and neuroendocaine cells in embryos are functioning in a primitive nervous system but that later in development, when the other neurones mature, their functional role is superceded by the more developed nervous and neuroendocrine systems. In the thymus gland, neurospecific protein is present in the same reticuloendothelial cells in the medullary centers as α_1-thymocin. Other neuroendocrine-like cells of circumventricular midline structures of the brain, where the blood-brain barrier is known to be absent, also have both nerve specific enolase and the non-neuronal form of the enzyme, indicating their developmental relation to neurones and possible neuroendocrine function. (Oertel, Schmechel)

Aldolase A, another enzyme which is not uniformly present in all cells, but is present in glia and some nerve cells. The differences in enzyme content suggest metabolic differences among neurones.

Two-dimensional electrophoresis has been used to separate the enzymes which are irreversibly inhibited with radioactivity labeled "Suicide Inhibitors". Glutamic acid decarboxylase (GAD) has been purified by similar techniques and an antibody to the enzyme obtained; the latter is being used to localize GAD in specific cells. (Oertel, Weise)

PUBLICATIONS:

Gilad, G.M. Gagnon, C., and Kopin, I.J.: Protein carboxymethylase activity in the rat superior cervical ganglion during development and after axonal injury. Brain Research (In press).

Gilad, G.M. and Kopin, I.J.: Neurochemical aspects of neuronal ontogenesis in the developing rat cerebellum: Changes in neurotransmitter and polyamine synthesizing enzymes: Journal of Neurochemistry (In press).

Gilad, G.M., Gilad, V.H. and Kopin, I.J.: The reaction of the mutant mouse nude to axonal injuries of the central nervous system: Experimental Neurology: (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00378-05 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Immunofluorescence Study of the Uptake of the Antibody to Dopamine-Beta-Hydroxylase in the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Michael A. Silver	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: .4	PROFESSIONAL: .3	OTHER: .1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A retrograde flow mapping technique that is specific for dopamine-β-hydroxylase (DβH) containing neurons, has recently been developed. This is based upon the phenomenon of specific uptake and retrograde flow of antibody to DβH (ADβH) by noradrenergic neurons. Following unilateral injection of ADβH into the locus coeruleus (LC), ADβH was immunocytochemically visualized within cell bodies of the contralateral LC, and ipsilateral A2 noradrenergic cell groups. Substantial numbers of cells were labelled bilaterally within the A1, A4 and A5 groups. This indicates that noradrenergic fibers arising from distant perikarya may innervate the noradrenergic neurons of the locus coeruleus.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The elucidation of the origin of adrenergic fibers terminating within the locus coeruleus.

Methods Employed: Immunohistochemical localization of ADβH by an indirect immunofluorescence technique utilizing a fluorescein-labelled antiserum to the primary antibody. The rats received stereotaxic injections of ADβH into the right caudal LC via a glass micropipette.

Major Findings: Following unilateral locus injection, ADβH was visualized within cell bodies of the contralateral LC, and ipsilateral A2 noradrenergic cell groups. Substantial numbers of cells were labelled bilaterally within the A1, A4 and A5 groups. No other cell bodies were visualized.

Significance to Biomedical Research and the Program of the Institute: This study represents the first indication that noradrenergic fibers arising from distant perikarya may innervate the noradrenergic neurons of the LC. This circuitry of noradrenergic projections may serve as the anatomical basis for a functional organization.

Publication:

Silver, M.A. and Jacobowitz, D.M.: Specific uptake and retrograde flow of antibody to dopamine-β-hydroxylase by central nervous system noradrenergic neurons *in vivo.* *Brain Res.*, 167: 65-75, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00382-05 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Mapping of Monoaminergic Neurons of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It was previously demonstrated that the nucleus tractus diagonalis exhibits cyclic changes in dopamine content over the estrus cycle, changes in dopamine turnover after ovarian hormones, and sex differences in dopamine that are apparently under the control of neonatal androgen. This region appears to be a site for complex interactions among several neurochemical systems in response to gonadal steroids. Neurochemical studies were carried out to determine the site of the dopamine cell body localization which projects to the nucleus of the tractus diagonalis. Stereotaxic lesion of the A-10 dopamine cell bodies resulted in an 84% decrease in the dopamine content of the nucleus tractus diagonalis thereby revealing the site of the dopaminergic input to this nucleus.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To map, biochemically, the dopaminergic cell body group that is responsible for innervation of the nucleus tractus diagonalis.

Methods Employed: 1) Bilateral stereotaxic lesion of the A-10 dopaminergic cell body group of the ventral tegmental area. 2) Microdissection of forebrain nuclei. 3) Microradioenzymatic assay of dopamine.

Major Findings: Areas which showed a significant decrease in dopamine concentration after bilateral destruction of the A-10 perikarya were the nucleus of the tractus diagonalis (84% ↓), nucleus accumbens (81% ↓), olfactory tubercle (76% ↓), lateral septum (65% ↓). No changes were observed in the periventricular and habenular nuclei.

Significance to Biomedical Research and the Program of the Institute: The nucleus of the tractus diagonalis is an important cholinergic nucleus of the brain which innervates the hippocampus, part of the cerebral cortex, amygdala, interpeduncular nucleus, ventral tegmental area and the substantia nigra zona compacta. Previous studies revealed that the nucleus tractus diagonalis exhibits cyclic changes in dopamine content over the estrus cycle, changes in dopamine turnover after ovarian hormones, and sex differences in dopamine that are apparently under the control of neonatal androgen. This region appears to be a site for complex interactions among several neurochemical systems in response to gonadal steroids. It is suggested that there is a dopaminergic-cholinergic link between the nucleus tractus diagonalis and the A-10 dopamine cell body groups.

Proposed Course of Project: Biochemical mapping of neurotransmitter systems and their neurochemical interactions in the brain will continue.

Publications:

Speciale, S.G., Crowley, W.R., O'Donohue, T.L. and Jacobowitz, D.M.: Forebrain catecholamine projections of the A5 cell group. Brain Res. 154: 128-133, 1978.

O'Donohue, T.L., Crowley, W.R. and Jacobowitz, D.M.: Biochemical mapping of the noradrenergic ventral bundle projection sites: Evidence for a noradrenergic-dopaminergic interaction. Brain Res., in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00388-03 LCS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neurochemical and Histochemical Studies of Cholinergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH
	Zehava Gottesfeld	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .8	PROFESSIONAL: .5	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the rat brain biochemical changes in choline acetyltransferase (CAT) following stereotaxic lesions revealed that cholinergic nerves of the nucleus of the tractus diagonalis project to the cingulate cortex, interpeduncular nucleus, ventral tegmental area, hippocampus, substantia nigra (zona compacta) and the lateral reticular nucleus. The nucleus tractus diagonalis is a major cholinergic nucleus that projects long descending neurons to regions of the forebrain, midbrain and hindbrain.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To investigate possible cholinergic projection sites of innervation from the nucleus tractus diagonalis of the rat brain.

Methods Employed: 1) Bilateral stereotaxic lesion of the nucleus tractus diagonalis. 2) Microdissection of brain nuclei. 3) Radioenzymatic assay for choline acetyltransferase (CAT).

Major Findings: 1) Following stereotaxic lesions of the nucleus tractus diagonalis significant decreases in CAT activity were found in the cingulate cortex, interpeduncular nucleus, ventral tegmental area, hippocampus, substantia nigra (zona compacta) and the lateral reticular nucleus.

Significance to Biomedical Research and the Program of the Institute: The nucleus tractus diagonalis is a nucleus that is known to project the cholinergic innervation to the hippocampus. This is the first realization that a major projection of cholinergic nerves emanates from this nucleus and projects to the midbrain and hindbrain. This is important for studies aimed at determining functions of acetylcholine in the brain.

Proposed Course of Project: Further possible projection sites will be sought and retrograde transport of horseradish peroxidase will be used to further elucidate projection sites of the nucleus tractus diagonalis.

Publications:

Gottesfeld, Z. and Jacobowitz, D.M.: Cholinergic projection of the diagonal band to the interpeduncular nucleus of the rat brain. Brain Res. 156: 329-332, 1978.

Hoover, D.B. and Jacobowitz, D.M.: Neurochemical and histochemical studies of the effect of a lesion of the nucleus cuneiformis on the cholinergic innervation of discrete areas of the rat brain. Brain Res., in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00389-03 LCS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Ovarian Hormones in Plasma Gonadotropins and on Serotonin Concentrations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Levels of serotonin were measured in microdissected, individual brain nuclei in ovariectomized rats after treatment with ovarian hormones. Regions sampled included nuclei in the forebrain, rostral and medial hypothalamus, and midbrain tegmentum. Estradiol benzoate decreased levels of luteinizing hormone in plasma but did not affect serotonin levels in any region. Progesterone alone elevated serotonin content in the nucleus tractus diagonalis and ventral tegmental area. The combined estrogen plus progesterone regime produced a surge in plasma luteinizing hormone and also markedly elevated serotonin in the median eminence.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the effects of ovarian hormones on serotonin concentrations in individual brain nuclei of ovariectomized female rats.

Methods Employed: 1) Microdissection technique to remove individual brain nuclei. 2) Enzymatic-isotopic microassay for serotonin. 3) Radioimmunoassay for luteinizing hormone.

Major Findings: Treatment of ovariectomized female rats with estrogen lowered plasma luteinizing hormone 54 hr later but had no effect on serotonin levels in any region. Treatment of estrogen primed rats with progesterone at 48 hr produced a surge in plasma luteinizing hormone and markedly elevated serotonin in the median eminence. Administration of progesterone alone had no effect on gonadotropin concentrations but elevated the serotonin content of the nucleus tractus diagonalis and ventral tegmental area.

Significance to Biomedical Research and the Program of the Institute: The present study demonstrates effects of ovarian hormones on central serotonergic systems in discrete regions of the brain. The results are relevant to the neural control of gonadotropin secretion, ovulation and mating behavior.

Publication:

Crowley, W.R., O'Donohue, T.L., Muth, E.A. and Jacobowitz, D.M.: Effects of ovarian hormones on levels of luteinizing hormone in plasma and on serotonin concentrations in discrete brain nuclei. Brain Res. Bull., in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00394-02 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Role of Central Cholinergic Mechanism in the Feedback Actions of Gonadal Steroids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

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SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.2	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The involvement of central cholinergic mechanisms in the feedback actions of gonadal hormones was examined by measuring choline acetyltransferase activity in rats (ChAT) and acetylcholine concentration (ACh) in microdissected brain nuclei after castration and gonadal hormone replacement. In males, castration resulted in increased plasma luteinizing hormone (LH), as well as in a) increased ChAT in the posteromedial amygdaloid nucleus; b) increased ACh in the ventral tegmental area and rostral nucleus tractus diagonalis; and c) increased ChAT and ACh in the medial preoptic nucleus. Testosterone attenuated all of these responses to castration, and also lowered ChAT in the rostral nucleus tractus diagonalis. Treatment of ovariectomized, estrogen-primed females with progesterone resulted in a surge in plasma LH, as well as in a) increased ChAT in the supraoptic nucleus; b) decreased ChAT in the caudal nucleus tractus diagonalis; c) decreased ACh in the ventral tegmental area; and d) decreased ChAT and ACh in the periventricular nucleus. The results demonstrate that cholinergic activity in several discrete brain regions known to be targets for testicular and ovarian hormones is altered by gonadectomy and gonadal hormone treatment. These brain areas may represent sites where circulating gonadal steroids initiate neuroendocrine feedback actions through cholinergic mediators.

Project Description:

Objectives: To anatomically identify and elucidate the role of central cholinergic neurons participating in the feedback actions of circulating gonadal hormones.

Methods Employed: 1) Surgical castration or ovariectomy. 2) Testosterone to males and estrogen and progesterone treatment to females. 3) Microwave irradiation of heads of animals used for ACh microassay. 4) Radioimmunoassay of LH. 5) Microdissection of brain nuclei. 6) Microenzymatic assays for ChAT and ACh.

Major Findings: 1) In male rats, lack of circulating testosterone due to castration appeared to stimulate cholinergic neurotransmission in the rostral nucleus tractus diagonalis, medial preoptic nucleus, ventral tegmental area, and posteromedial amygdaloid nucleus concomitant with increased LH secretion. The results suggest that testosterone affects behavior via cholinergic mechanisms in these areas, and that they may represent neural substrates for the observed effects of centrally-acting cholinomimetic and anticholinergic drugs on LH secretion. 2) In ovariectomized female rats, estrogen priming followed by progesterone appeared to depress cholinergic activity in the caudal nucleus tractus diagonalis, periventricular nucleus, and ventral tegmental area at a time when LH secretion was stimulated. The results suggest that cholinergic neurons in these areas are inhibitory to gonadotropin release, which is consistent with the nicotinic-cholinergic inhibitory system suggested by pharmacologic studies. Cholinergic activity in the supraoptic nucleus of the same female rats was increased by estrogen + progesterone, suggesting that cholinergic stimulation of vasopressin neurosecretion is active during the LH surge. Previous work by this and other laboratories has suggested that vasopressin secretion is in fact elevated at this time.

Significance to Biomedical Research and the Program of the Institute: This study provides insight into how circulating hormones may affect behavior by elaborating their effects at the level of the individual nuclei of the brain. At the same time, it provides information about the functional anatomy of central cholinergic systems pertinent to the control of gonadotropin secretion. This knowledge will prove useful in understanding the mechanics of brain-endocrine interactions and their perturbation by pharmacological agents or pathological states.

Proposed Course of Project: 1) The effects of discrete lesions and stereotaxic drug implantations on LH secretion will be examined to clarify the functional significance of cholinergic neurons in these brain areas. 2) Cholinergic interactions with other putative neurotransmitter systems, particularly dopamine and luteinizing hormone-releasing hormone, will be studied in an effort to determine the specific role of ACh neurons in the feedback effects of gonadal hormones.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00395-01 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on the CNS Cholinergic Neurons in the Spontaneously Hypertensive Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The choline acetyltransferase (ChAT) activity was measured in numerous discrete brain nuclei of 4, 8, and 12 wk old spontaneously hypertensive rats (SHR) and their age matched Wistar Kyoto control rats (WKY). The acetylcholine (ACh) concentration was also measured in hindbrain nuclei of 12 wk old SHR and WKY. Changes in ChAT activity of several nuclei were observed in SHR of all 3 age groups. The greatest alteration (79% increase) was found in the ChAT activity of the locus coeruleus of 12 wk old SHR. A 31% increase in ACh was also detected. No differences were found in this nucleus in 4 or 8 wk SHR. Other nuclei known to be involved in blood pressure control which showed differences in ChAT activity in SHR compared to WKY were the paraventricular nucleus, dorsomedial nucleus of the hypothalamus, posterior hypothalamus and the nucleus reticularis gigantocellularis. A significant increase was also found in the anterior ventral thalamus in 4 and 8 wk SHR.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) Recent pharmacological studies have demonstrated that CNS cholinergic mechanisms may be involved in blood pressure control. The present study is an attempt to determine whether biochemical differences in cholinergic neurons of discrete brain areas exist between 4, 8, and 12 wk SHR and WKY.

Methods Employed: 1) Indirect tail-cuff measurement of systolic blood pressure. 2) Microdissection technique for removal of discrete nuclei. 3) Radioisotopic assay for choline acetyltransferase and acetylcholine.

Major Findings: 1) There was a significant elevation of the ChAT activity and ACh content of the locus coeruleus of 12 wk old SHR with established hypertension but not in those SHR which had not yet developed hypertension (4 wk) or were in the developing stages (8 wk). 2) Other brain nuclei known to be associated with cardiovascular control showed alterations in ChAT activity in SHR.

Significance to Biomedical Research and the Program of the Institute: Evidence has been provided that CNS cholinergic neurons are altered in this model of hypertension. This is a starting point for studies aimed at determining the role of CNS cholinergic systems in the development and maintenance of hypertension in animal models and in human essential hypertension.

Proposed Course of Project: The next phase of this study will be to determine the significance of the alterations in ChAT activity and ACh content of the locus coeruleus in the 12 wk SHR. This will be accomplished by investigating the interaction of ChAT in the locus coeruleus with the noradrenergic cell bodies in this nucleus. Studies will also be performed to determine the effects of locus coeruleus lesions in the development of hypertension in the SHR.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00396-01 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on Substance P as the Neurotransmitter of Baro- and Chemoreceptor Afferents in the Nucleus Tractus Solitarius

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Richard A. Gillis	Dept. Pharmacology	Georgetown Univ.	
	Betty Hamilton	Dept. Anatomy	Georgetown Univ.	
	West Norman	Dept. Anatomy	Georgetown Univ.	

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hypothesis that substance P is the neurotransmitter of baro- and chemoreceptor nerve terminals synapsing in the nucleus tractus solitarius was tested by 1) measuring the content of substance P in four regions of NTS and in the sensory ganglia containing cell bodies of baro- and chemoreceptor afferents, and 2) determining the effect of intracranial sectioning of the rootlets of the IX and Xth nerves on the substance P content of the four regions of the NTS. Measurement of substance P in NTS and sensory ganglia revealed a high level of this material in parts of NTS where baro- and chemoreceptor afferents synapse, and in nodose and petrosal ganglia. Denervation of cranial nerves IX and X of cats resulted in a 50% reduction of substance P content of parts of NTS where these afferents terminate, but no significant reduction in substance P content of parts of the NTS not involved in regulation of cardiovascular function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Various investigators using immunohistochemical techniques have reported substance P like immunoreactivity in the NTS and fibers entering the brain stem via the glossopharyngeal nerve. In addition, one of the sensory ganglia-containing cell bodies of baro- and chemoreceptor afferent fibers (the nodose ganglion) has been described as containing substance P immunoreactive cell bodies. Because baro- and chemoreceptor afferents are known to terminate in the NTS the purpose of this study was to determine whether the primary afferent input to the NTS from baro- and chemoreceptors is via substance P neurons.

Methods Employed: 1) Chronic intracranial deafferentation of the IX and Xth cranial nerves of cats. 2) Microdissection technique for removal of discrete nuclei. 3) Radioimmunoassay of substance P.

Major Findings: 1) Substance P like-immunoreactivity was found in the NTS and in the nodose and petrosal ganglia. 2) Denervation of cranial nerves IX and X resulted in a 50% decrease of the substance P content of parts of NTS where baro- and chemoreceptor afferents terminate.

Significance to Biomedical Research and the Program of the Institute: These data suggest that substance P is a neurotransmitter in baro- and chemoreceptor afferents in NTS. This is an important finding in terms of delineating the neurotransmitters in CNS neurons governing cardiovascular reflex activity.

Proposed Course of Project: 1) Additional neuroanatomical studies to confirm these data. 2) Pharmacological studies to investigate the effects of substance P in the NTS on cardiovascular reflexes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00397-01 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of α-Melanocyte Stimulating Hormone in Brain Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Nguyen B. Thoa	Pharmacologist	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Clivel G. Charlton	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

Office of the Chief

LAB/BRANCH Laboratory of Clinical Science

SECTION Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using an immunocytochemical approach, an extensive system of neurons containing α-melanocyte stimulating hormone (α-MSH) was identified and mapped in the rat brain. α-MSH throughout the rat brain was determined by combining microdissection techniques with a radioimmunoassay. The immunoreactive compound in brain was characterized by high pressure liquid chromatography combined with radioimmunoassay. The major immunoreactive component has an identical retention time as standard α-MSH, suggesting chemical identity of the peptides. Lesion of the arcuate nucleus, the site of α-MSH containing perikarya, depleted α-MSH throughout the brain. α-MSH in the pineal gland and several brain regions investigated demonstrated a marked circadian rhythm. α-MSH has also been identified in human post mortem specimens with a regional distribution similar to that of rat and cat brain. α-MSH immunoreactive has also been identified in rat and human cerebrospinal fluid (CSF). Results suggest that α-MSH concentrations in rat CSF may provide an index for measuring α-MSH release in vivo. A calcium dependent in vitro α-MSH release has been demonstrated using slices of fresh rat hypothalami.

Project Description:

Objectives: Identification, characterization, stereotaxic mapping and determination of the role of α-MSH in brain neurons.

Methods Employed: 1) Development of antibodies of α-MSH. 2) Radioimmunoassay of α-MSH. 3) Immunocytochemistry of α-MSH. 4) Microdissection of rat and cat brain nuclei. 5) Development of a microdissection technique for human brain tissue. 6) Stereotaxic neurosurgery of the rat brain. 7) Push-pull cannulae to remove rat cerebrospinal fluid. 8) Release from hypothalamic slices. 9) High pressure liquid chromatography.

Major Findings: 1) Identification and characterization of α-MSH in the brain. α-MSH was identified and characterized in rat brain by combining high pressure liquid chromatography (HPLC) with a sensitive radioimmunoassay. The major immunoreactive component in rat brain has an identical HPLC retention time as standard α-MSH. This data suggests chemical identity between α-MSH and the major immunoreactive compound in brain. The HPLC was also able to resolve two minor immunoreactive components in rat brain which have similar immunoreactivity and retention times as standard α-MSH. 2) Distribution of α-MSH in the brain. α-MSH-like immunoreactivity was contained in fibers throughout the rat and cat brain. The distribution of α-MSH was determined by immunocytochemistry as well as by radioimmunoassay combined with microdissection techniques. High concentrations of α-MSH were contained in the nucleus interstitialis stria terminalis, the median eminence and the medial preoptic, anterior hypothalamic, periventricular, paraventricular, arcuate, dorsomedial, and posterior hypothalamic nuclei. Moderate α-MSH concentrations were noted in the amygdala, septum, central gray dorsal raphe, and the nucleus tractus solitarius. Cell bodies containing α-MSH were observed only in the arcuate nucleus. Lesion of the rat arcuate nucleus produced a marked decrease in α-MSH in major terminal regions of the brain suggesting that this region is the source of α-MSH in the brain. Consistent with this finding, hypophysectomy does not cause a decrease in the number of α-MSH containing nerves in the brain. 3) Circadian rhythm of α-MSH. A significant circadian variation in α-MSH was observed in several discrete regions of the rat brain. During the 12 hour light: 12 hour dark cycle, α-MSH concentrations, in each case, were highest during the light period and lowest during the dark period. At 0900, three hours after lights were turned on, the peak α-MSH concentration occured in the median eminence, the arcuate, and the dorsomedial and thalamic periventricular nuclei. The paraventricular and anterior hypothalamic nuclei had highest α-MSH concentrations at 1300. In the medial preoptic nucleus, the peak α-MSH concentrations appeared at 1700. A diurnal rhythm of α-MSH was observed in the pineal gland with peak concentrations at 0700 hours. This rhythm persisted in hypophysectomized and superior cervical ganglionectomized rats. Animals maintained in chronic light for seven days had a decrease in amplitude of the α-MSH rhythm. Animals maintained in chronic dark, however, still maintained an α-MSH rhythm in the pineal but the peak shifted to 0500 and was greater in magnitude than the 0700 peak of control animals. 4) Identification of α-MSH in human brain and cerebrospinal fluid (CSF). Human post mortem brain specimens were obtained and microdissected. The human brain had concentrations of α-MSH quantitatively and qualitatively similar to the rat and cat brain. Human CSF also had measurable immunoreactive α-MSH. Concentrations ranged from 4.0 to 73.3 pg/ml with a mean of 22.9 pg/ml. α-MSH has also been measured in the cerebrospinal fluid of rats. To measure the in vivo release of α-MSH, rats were perfused through the lateral ventricle with artificial CSF and fractions were collected from the cisterna magna and measured for α-MSH immunoreactivity. The results indicate a high turnover rate for brain α-MSH. 5) In vitro release of α-MSH. α-MSH release was stimulated from slices of fresh rat hypothalamus using high concentrations of potassium. Potassium stimulation in the absence of calcium also resulted in α-MSH release but was significantly attenuated compared to release in the presence of calcium.

Significance to Biomedical Research and the Program of the Institute: The present studies demonstrate the existence of α-MSH in human and rat brain and cerebrospinal fluid. The basic anatomical, and physiological neurochemical studies reported here lay the groundwork for a rational approach in studying the role of α-MSH in central nervous system physiology and pathophysiology.

Proposed course of the project: Further studies are in progress to determine the transsynaptic actions of α-MSH and influence on physiological and psychological processes. α-MSH concentrations will be determined in the CSF of patients with various neurological and psychological disorders to examine a potential role for this peptide in neuropathology.

Publications:

Jacobowitz, D.M., and O'Donohue, T.L.: α-Melanocyte stimulating hormone: Immunohistochemical identification and mapping in neurons of the rat brain. Proc. Natl. Acad. Sci. USA 75: 6300-6304, 1978.

O'Donohue, T.L., Miller, R.L., and Jacobowitz, D.M.: Identification, characterization and stereotaxic mapping of intraneuronal α-melanocyte stimulating hormone-like immunoreactive peptides in discrete regions of the rat brain. Brain Res., in press, 1979.

O'Donohue, T.L., Miller, R.L., Pendleton, R.C., and Jacobowitz, D.M.: A diurnal rhythm of immunoreactive α-melanocyte stimulating hormone in discrete regions of the rat brain. Neuroendocrinology, in press, 1979.

O'Donohue, T.L. and Jacobowitz, D.M.: In Neuroactive Compounds and Their Cell Receptors. Basel, Karger Press, 1979.

O'Donohue, T.L., Miller, R.L., Pendleton, R.C. and Jacobowitz, D.M.: Demonstration of circadian rhythm of α-melanocyte stimulating hormone in the rat pineal gland. Brain Res., in press, 1979.

O'Donohue, T.L., Holmquist, G.E., and Jacobowitz, D.M.: Effect of hypophysectomy on α-melanotropin concentrations in discrete regions of the rat brain. Neuroscience Letters, in press, 1979.

O'Donohue, T.L. and Jacobowitz, D.M.: Studies of α-melanotropin in the rat brain. Polypeptide Hormones, 12th Miles International Symposium, in press, 1979.

O'Donohue, T.L., Charlton, C.G., Helke, C.J., Massari, V.J., Tizabi, Y., Miller, R.C. and Jacobowitz, D.M.: Identification of α-melanocyte stimulating hormone immunoreactivity in rat and human brain and cerebrospinal fluid. Proceedings of the Sixth American Peptide Symposium, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00398-01 LCS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Correlation of Aggressivity with Neurotransmitters in Discrete Brain Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS NIMH
	Yousef Tizabi	Department of Pharm.	Howard Univ.
	V. John Massari	Department of Pharm.	Howard Univ.
	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Gertrude D. Maengwyn-Davies	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)

Office of the Chief
Section on Medicine

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .4	PROFESSIONAL: .3	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) An aggressive strain of mice has a higher steady-state concentration as well as turnover of catecholamine in the frontal cortex, caudate nucleus and hypothalamus when compared to a docile strain, suggesting that neuronal activity in these areas may be greater in aggressive mice. 2) Norepinephrine and dopamine levels and turnover were measured in 17 discrete brain regions of Swiss-Webster (NIH) mice made aggressive by prolonged isolation. Isolated non-fighters were used as controls. The norepinephrine steady-state level was significantly lower in olfactory tubercle and substantia nigra and significantly higher in the septal area of the aggressive mice. Norepinephrine turnover was only higher in the A-10 region (ventral tegmental area) of the aggressors. Dopamine steady-state level and turnover was lower in olfactory tubercle and higher in caudate putamen of the aggressors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) Attempt to correlate genetic aggressivity with catecholamine dynamics in discrete brain regions of mice. 2) To correlate catecholamine variations in discrete brain areas with the aggressive component of the "isolation syndrome" in mice.

Methods Employed: 1) Radioenzymatic assay for catecholamine. 2) Microdissection technique to remove individual brain nuclei. 3) Isolation of mice in individual cages for 6 weeks and use of isolated non-fighters as controls for the isolated fighters (aggressor mice).

Major Findings: 1) In general, amine levels are comparable in brains of mice and rats. The more aggressive strain has a higher level of norepinephrine in the frontal cortex, nucleus accumbens, caudate nucleus, septal area, medial and lateral preoptic nucleus, hippocampus and entorhinal cortex and greater concentration of dopamine in the frontal cortex, nucleus accumbens, olfactory tubercle, caudate nucleus and hypothalamus. The decrease in catecholamine concentration following alpha-methyl-para-tyrosine injection is not uniform in different brain regions. 2) Relatively few areas show a change in catecholamine concentration or turnover in association with the aggressive component of isolation. Isolation-induced aggression is correlated with an increased NE turnover in the A-10 DA cell body region which suggests an inhibitory interaction between NE and DA at this level and is subsequently reflected in decreased DA levels and turnover in the olfactory tubercle. Similarly, a decrease in steady state levels of NE in the substantia nigra is reflected in an increase in DA turnover in the striatum.

Significance to Biomedical Research and the Program of the Institute: 1) This study demonstrates a correlation between catecholamine dynamics and aggressive tendency in mice. It shows that genetic differences in steady-state levels and turnover of catecholamine may be apparent in only a limited number of brain regions. 2) The changes in the steady state level and turnover of DA in the caudate putamen and olfactory tubercle suggests the involvement of DA in the motor function associated with isolation-induced aggression, as well as the involvement of the olfactory mechanism in aggressive behavior.

Proposed Course of Project: Investigate the involvement of other neurotransmitters and peptides in aggressive behavior.

Publication:

Tizabi, Y., Thoa, N.B., Maengwyn-Davies, G.D., Kopin, I.J., and Jacobowitz, D.M.: Behavioral correlation of catecholamine concentration and turnover in discrete brain areas of three strains of mice. Brain Res., 166: 199-205, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00399-01 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neurochemical and Immunochemical Studies of Peptidergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Terry W. Moody	Guest Worker	BP	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Nguyen B. Thoa	Pharmacologist	LCS	NIMH

COOPERATING UNITS (if any)

Office of the Chief, LCS
Biological Psychiatry Branch

LAB/BRANCH Laboratory of Clinical Science

SECTION Histopharmacology

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) An antiserum for bombesin, a tetradecapeptide, was used to map the regional distribution of bombesin-like immunoreactivity (BLI) in the rat brain. Using immunocytochemical techniques, BLI was observed in discrete varicose nerve fibers. Cell bodies were observed in the preoptic, hypothalamic and mammillary nuclei and in the mesencephalon. Using a radioimmunoassay combined with microdissection methods, BLI was observed to be 30-fold greater in high regions than low regions. When brain extracts were fractionated using high-pressure liquid chromatography, one major peak of immunoreactivity was obtained which was distinct from substance P. Release of bombesin-like peptides from rat hypothalamic slices was investigated. 2) Immunocytochemical studies of the opiate ligand, enkephalin, revealed the distribution of a population of large axonal-like processes throughout the rat forebrain. The mapping of the distribution of these axonal-like processes, in addition to perikarya of the forebrain, was undertaken.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) Identification, characterization and stereotaxic mapping of intraneuronal bombesin-like immunoreactivity (BLI) in the brain. 2) Mapping of axonal processes and cell bodies of leu enkephalin-containing neurons of the brain.

Methods Employed: 1) Radioimmunoassay for bombesin. 2) Immunocytochemistry. 3) Microdissection of rat brain nuclei. 4) High pressure liquid chromatography. 5) Release from hypothalamic slices.

Major Findings: 1) Endogenous bombesin-like peptides were detected by immunocytochemical techniques as well as by radioimmunoassay combined with microdissection techniques. BLI was observed in discrete varicose nerve fibers. The concentration of BLI was 30 fold greater in high regions (n. tractus soltarius, interpeduncular n., and substantia gelatinosa) than low regions (caudate, hippocampus and cingulate cortex). Following vinblastine treatment, cell bodies were observed in the preoptic, hypothalamic and mammillary nuclei and in the mesencephalon (central grey, colliculi and reticular formation). The distribution of BLI and substance P is strikingly coincidental; however, radioimmunoassay analysis of HPLC fractions of brain extracts indicates that the two peptides are distinct. The release of bombesin-like peptides from rat hypothalamic slices was investigated. The amount released increased 2-3 fold when the brain slices were treated with depolarizing stimuli such as K^+ or veratridine in the presence of Ca^{++}. 2) A large area of enkephalin-containing axons was observed just ventral to the globus pallidus, in the supraoptic commissure, ansa lenticularis, stria terminalis and median forebrain fundle. Enkephalin cell bodies were observed in the hypothalamus, (dorsomedial, paraventricular, periventricular, ventromedial and arcuate nuclei), preoptic area, bed nucleus of the stria terminalis, septal area, nucleus accumbens and the medial caudate nucleus.

Significance to Biomedical Research and the Program of the Institute: In the CNS bombesin induces hypothermia, analgesia, and hyperglycemia. The extensive distribution of bombesin-like peptides in the brain which are capable of being released by depolarizing stimuli suggests that it is involved in significant neuronal circuitry, supports the notion of a neuroregulatory role for this neuropeptide, and lays the groundwork for a rational approach for further study of possible interactions of bombesin with other neuronal systems.

The widespread population of enkephalinergic cell bodies in the hypothalamus, preoptic and septal areas is consistent with the interpretation that enkephalin is generally contained within interneurons. A large cluster of cell bodies was found in the lateral part of the ventromedial nucleus which is the satiety center. Stimulation of this area results in reduced eating. In this regard it is interesting that morphine addicts show a marked reduction in appetite.

Proposed Course of Project: Follow-up studies include further mapping of bombesin in the brain and measurement in post-mortem human brains.

Publication:

Jacobowitz, D.M., Silver, M.A. and Soden, W.G.: Mapping of leu enkephalin-containing axons and cell bodies of the rat forebrain. In Usdin, E. and Bunney, W., McMillan, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00351-05 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	M. A. Elchisak	Guest Worker	LCS NIMH
	C. R. Lake	Staff Psychiatrist	LCS NIMH
	E. Gordon	Head, Unit on Clinical Biochemistry	LCS NIMH
	R. Polinsky	Staff Physician	LCS NIMH
	R. S. Burns	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Therapeutics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to develop techniques for studying central nervous system monamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. Studies of the kinetics and clearance of deuterated homovanillic acid (HVA) from the circulation were conducted in rhesus monkeys and patients. In primates, calculated basal rate of HVA production by the entire animal was 5.2-12.6 mg/kg/hr. Less than one percent of HVA in CSF is derived from blood, and HVA in urine is derived only from blood, not from renal metabolism of dopamine. Infusion of probenecid indicated that approximately 70% of total body HVA originated in the CNS. An assay for dopamine sulfate has been established. Studies are in progress on pharmacological effects on CSF norepinephrine. Plasma levels, pharmacokinetics, and renal clearance of phencyclidine (PCP) are under investigation.

Project Description:

Objectives: 1. To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. 2. To carry out pharmacokinetic studies of psychoactive drugs. 3. To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed: 1. Biochemical methods: Flourometric, gas chromotographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radio-immunoassay techniques are used for assay of drugs in body fluids (probenecid and d-amphetamine). 2. Physiological methods: As the project progresses. an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical *in vivo* experiments on brain metabolism, a small primate metabolic laboratory has been developed with the capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired rhesus monkeys for days or weeks at a time.

Major Findings: Work has continued on the development of a technique to estimate the rate of homovanillic acid (HVA) production in human brain from examination of blood HVA (Drs. Elchisak, Polinsky, Ebert, Kopin). We hope that this will provide an assessment of brain dopamine metabolism in man. This technique requires complete information about the kinetics of HVA elimination and distribution both before and after administration of probenecid. This was examined in detail in the Rhesus monkey (macaca mulatta). HVA labelled with two deuterium atoms (d_2-HVA) was used to label the peripheral body pool of endogenous HVA (d_0-HVA). After rapid intravenous injection of d_2-HVA, the concentrations of both d_2- and d_0-HVA were determined by gas chromatography-mass spectrometry in sequential samples of blood, serum, and urine. The decline of serum d_2-HVA concentration with time was multiexponential, with a biological half-life ranging from 35.9 to 102 min. The apparent volume of distribution was 0.813-1.17 l/kg. In most animals, only about 50% of the administered dose of d_2-HVA was recovered unchanged in the urine. and renal clearance was 3.79-17.0 ml/kg/min. The calculated basal rate of HVA production by the whole body was 5.23-12.6 ug/kg/hr. Results after intravenous infusion of d_2-HVA indicate that less than one percent of HVA in lumbar or ventricular CSF is derived from blood. HVA in urine is derived only from blood, not from renal metabolism of dopamine (Elchisak, Polinsky, Ebert, Kopin).

During probenecid infusion in the Rhesus monkey, the apparent rate of HVA production by the body was increased. This was demonstrated to be due to a decrease in the apparent volume of distribution of HVA after probenecid. Preliminary results, after considering the volume of distribution change, indicate that approximately 70 percent of the total body HVA produced in the Rhesus monkey is derived from the brain.

Preliminary data suggests that the kinetics of HVA elimination and distribution in humans is similar to that in monkeys (Elchisak, Polinsky, Ebert, Zavadil, Kopin). Probenecid does not appear to change the apparent volume of distribution of HVA in humans, but this must be more carefully evaluated. The analytical assay for HVA has been improved, resulting in a lower limit of detection of less than 100 picograms of HVA per sample (Elchisak). This should easily enable us to complete the development of the method to estimate the rate of HVA production in the brain of humans from blood sampling only. We can then utilize this method to evaluate the central effects of psychoactive drugs in humans and, possibly, to demonstrate defects of the central dopaminergic system in certain disease states.

Investigation to determine the metabolic fate of d_2-HVA in the body has also been initiated, since only 50% of the compound appears unchanged in the urine. Preliminary results indicate that conjugation is only a minor pathway of HVA metabolism. Elimination in the feces is also minor (Cadet, Elchisak, Ebert).

Dopamine sulfate ($DASO_4$) is a metabolite of dopamine which occurs in several animal species. Work has been initiated to demonstrate the occurrence of this compound in brain and body fluids of primates, including humans. Methods adapted from the literature were used to synthesize and separate three different ^{14}C-labelled isotopes of $DASO_4$: dopamine-3-0-sulfate, dopamine-4-0-sulfate, and dopamine-3, 4-disulfate (Elchisak, Powers, and Ebert). The radio-labelled standards were then used to determine optimal conditions for hydrolysis of $DASO_4$ to free DA, as well as to develop procedures for the isolation of $DASO_4$ from body fluids. Currently, $DASO_4$ is being hydrolyzed and measured as free dopamine. A method utilizing gas chromatography-mass spectrometry has been adapted from the literature to quantitate dopamine from tissues or fluids, and modified to detect approximately 25-50 picograms of dopamine per sample. $DASO_4$ has been detected in ventricular and lumbar CSF of Rhesus monkeys, as well as blood and urine of both monkeys and humans. The concentrations of $DASO_4$ are substantially greater than those of free dopamine in all these fluids. Evaluation of the significance of $DASO_4$ as a central metabolite of dopamine will continue during the next year.

Studies have continued on the physiological significance of CSF norepinephrine (NE) levels using lateral ventricle CSF collected chronically from Rhesus monkeys. A dose response study of CSF NE to doses of d- and l-amphetamine ranging from 0.1-1.0 mg/kg demonstrated dose related increases in CSF NE that were not significantly different between the stereoisomers. A dose of 1.0 mg/kg of d-amphetamine produced a four-fold increase in CSF NE which remained elevated for 24 hours (Ziegler, Lake, Ebert). A similar study was performed during acute and chronic imipramine treatment of Rhesus monkeys (Major, Ziegler, Ebert). A significant increase occurs in CSF NE acutely after administration of imipramine, which decreases with chronic treatment, and increases again briefly upon cessation of treatment. Both of these studies give additional evidence that CSF NE reflects the release of NE in the brain.

Studies of the pharmacokinetics of amphetamine in hyperactive children and the relationship of blood levels of amphetamine to clinical response have continued. A series of studies of the acute administration of 0.5 mg/kg of d-amphetamine in tablet, elixir, and sustained release form have concluded. Both behavioral and motor activity responses as analyzed by differences between amphetamine and placebo days indicate significant responses to amphetamine between hours 1-4. These responses occur primarily during the absorption phase and do not correlate with specific plasma amphetamine levels. A complete discussion of these projects is found in Projects Z01 MH 00100-04 AP and Z01 MH 00040-03 AP of the Biological Psychiatry Branch.

Phencyclidine (PCP) and structurally related compounds represent a unique drug class. The time course of the psychosis and neurological sequellae following chronic abuse of the drug may be related to its elimination kinetics. A GC-MS assay for PCP and its metabolites has been established in the laboratory (Burns, Hawks (NIDA), Ebert). A pilot study of the kinetics of PCP and its metabolites in primates is being carried out. The effect of urinary acidification on the renal clearance of PCP is being studied in rats.

Significance to Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods for determining central nervous amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders and assessing the effects of drugs on the amines in the brain.

Proposed Course: The techniques described above are now being applied to studies in patients. When appropriate, some of the above techniques will also be studied next year in normal volunteers in order to generate a comparison group to patients with various disorders of neurotransmission.

Publications:

1. Elchisak, M.A., Polinsky, R.J., Ebert, M.H., Powers, K.J., and Kopin, I.J.: Contribution of plasma homovanillic acid (HVA) to urine and cerebrospinal fluid HVA in the monkey and its pharmacokinetic disposition. Life Sciences 23: 2339-2348, 1978.

2. Elchisak, M.A., Polinsky, R.J., Ebert, M.H., Modlin, L.T., and Kopin, I.J.: Kinetics of homovanillic acid and determination of its production rate in the Rhesus monkey. Life Sciences 24: 1493-1502, 1979.

3. Brown, G.L., Ebert, M.H., and Hunt, R.D.: Plasma d-amphetamine absorption and elimination in hyperactive childrn. Psychopharmacology Bulletin 14: 33-35, 1978.

4. Brown, G.L., Hunt, R.D., Ebert, M.H., Bunney, W.I., and Kopin, I.J.: Plasma levels of d-amphetamine in hyperactive children. Psychopharmacology 62: 133-140, 1979.

Publications (Continued)

5. Ziegler, M.G., Lake, C.R., and Ebert, M.H.: Norepinephrine elevations in cerebrospinal fluid after d- and l-amphetamine. European Journal of Pharmacology 57: 1979.

6. Powers, K.H. and Ebert, M.H.: Comparison of RIA and GC-MS assays. Biomedical Mass Spectrometry, in press.

7. Mudd, S.H., Ebert, M.H., and Scriver, C.R.: Labile methyl group balances in the human: the role of sarcosine. Metabolism, in press.

8. Ebert, M.H., Cowdry, R., Post, R., and Goodwin, F.R. Cerebrospinal fluid probenecid levels in the oral probenecid test. Psychopharmacology, in press.

9. Ziegler, M.G., Lake, C.R., Wood, J.H., and Ebert, M.H.: Norepinephrine in cerebrospinal fluid: basic studies, effects of drugs and disease. In Neurobiology of Cerebrospinal Fluid. New York, Plenum Press, 1979, in press.

10. Ebert, M.H., Kartzinel, R., Cowdry, R.W., and Goodwin, F.K.: Cerebrospinal fluid amine metabolites and the probenecid test. In Wood, J. (Ed.): Neurobiology of Cerebrospinal Fluid. New York, Plenum Press, 1979, in press.

11. Brown, G.L., Ebert, M.H., Mikkelsen, E.J., and Hunt, R.D.: Clinical pharmacology of d-amphetamine in hyperactive children: In Pharmacokinetics and Clinical Response, Gottschalk, L.A., Spectrum Publications, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00352-04 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	R. Polinsky	Staff Physician	LCS NIMH
	W. H. Kaye	Staff Psychiatrist	LCS NIMH
	H. Gross	Staff Psychiatrist (Medical Officer)	FDA
	H. Weingartner	Acting Chief, LPP	LPP NIMH
	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several neuropsychiatric syndromes including Altzheimer's dementia, Gilles de la Tourette's syndrome are being studied in terms of memory function, neuroendocrine function, and possible pharmacological treatment. The pathophysiology and neuropharmacology of anorexia nervosa and orthostatic hypotension is also under investigation. The effects of arecholine (a cholinergic receptor agonist), and lecithin (a dietary source of choline) on memory function in Altzheimer's dementia is under study. The conclusion of a trial of lithium carbonate in the treatment of anorexia nervosa indicated that it was not efficacious. A family study of Gilles de la Tourette's syndrome suggests that the patients subdivide by clinical criteria into three groups, one with a family history of Tourette's syndrome. Family history was significantly related to the occurence of sleep disturbance, obsessive compulsive behavior, and positive haloperidol response.

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neuroendocrine function, neurotransmitter metabolism, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Altzheimer's dementia. Other diseases under intensive study are Gilles de la Tourette's syndrome, anorexia nervosa, and orthostatic hypotension.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short term memory; (2) long term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes; and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory. The procedures to measure short term memory functions include immediate recall of random high and low imagery words, consonant trigrams, taxonomically organized words, digits, and forms generated from mutually exclusive binary dimensions. Long term memory functions are tested by using equivalent sets of stimuli with varying periods of delay. Encoding strategies associated with normal versus disturbed brain states is measured using sorting tasks having redundant and random word stimuli.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of varying etiologies, focusing on the adrenergic and cholinergic systems.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses one can infer the functional state of dopaminergic transmission in the tuberoinfundibular systems. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double blind design, appropriate rating scales, placebo controls. Methods used in studies of neurotransmitter metabolism are those methods outlined in Project Z01 MH 00351-05 LCS.

Major Findings: Pharmacological studies of memory function have continued this year in collaboration with Drs. Weingartner, Sitaram, and Gillin. Parameters of memory processing and retrieval that were previously documented in Huntington's chorea are now being studied in a series of patients with Altzheimer's dementia and Korsakoff's psychosis. When concluded, these studies will indicate the specificity of the processing and retrieval changes that we have documented in these several dementias. A clinical trial of acute administration of arecholine (a cholinergic receptor agonist), lecithin (a dietary source of choline), and amphetamine in Altzheimer's dementia and Korsakoff's psychosis is underway. Clinical neurochemical studies of norepinephrine, dopamine, and serotonin metabolism are also underway in these dementias (Drs. Kaye and Ebert).

The clinical research program in primary anorexia nervosa has continued this year (Drs. Kaye, Gross, & Ebert). Clinical trials of lithium carbonate and delta-9-tetrahydrocannabinol have concluded. A variety of neuroendocine and neurochemical studies are in progress to further delineate the pathophysiology of the anorexia nervosa syndrome. Lithium carbonate was studied in a prospective, double blind, parallel design trial of 8 lithium and 8 placebo treated patients. The trial lasted 4 weeks and both groups received behavior modification treatment. There was a statistically significant gain of weight over time in both groups, but the effect of lithium was not significant. With regard to psychometrics, a number of anorexic behaviors and mood items improved over time significantly, but there was no evidence of a significant drug effect. In collaboration with Drs. Wachslicht-Rodbard and Roth, a study of insulin receptors in anorexia nervosa has concluded. Untreated patients showed increased insulin binding to receptors on erythrocytes (mean ± S.E.M., 12.2 ± .99 per cent of total); after weight gain, these increased levels returned to normal (6.8 ± .42 vs. 6.7 ± .63 per cent of total). Increased binding was due to an increased number of receptors per cell, with little or no change in receptor affinity. In 5 patients, results of insulin binding to erythrocytes correlated closely with results obtained with monocytes, suggesting that these insulin receptor changes are taking place in other tissues as well. Several neuroendocrine protocols are in progress to further delineate the hypothalamic dysfunction that occurs in acute anorexia nervosa. These include CSF and plasma vasopressin levels after hypertonic saline infusion, plasma and urinary melatonin rhythm, and prolactin and luteinizing hormone responses after dopamine infusion (Drs. Gold and Lewy). Initial data from intravenous probenecid tests in patients acutely ill and recovered indicate decreased serotonin and dopamine synthesis in the central nervous system when the patients are ill.

Studies of Gilles de la Tourette's syndrome have included the conclusion of two therapeutic drug trials and a continuation of family studies (Drs. Polinsky, Nee, Caine, Ebert). A therapeutic trial of lecithin is beginning. Among the first 50 patients studied, 16 patients had a family history for Tourette's syndrome, and another 16 had a family history for tics. Twenty-four families had more than two members with Tourette's syndrome or tics. Among the 50 patients there was a high frequency of sleep disturbance, learning disability, self-destructive behavior, inappropriate sexual activity, and antisocial behavior. Family history was significantly related to the occurrence of sleep disturbance, obsessive-compulsive behavior, and positive haloperidol response. The precise mode of genetic transmission in familial Tourette's syndrome remains to be determined. A double blind, placebo controlled trial of clozapine (a neuroleptic which has not been reported to cause tardive dyskinesia) was not efficacious in the treatment of Tourette's syndrome. A double blind crossover trial of chlorimipramine and desmethylimipramine in Tourette's syndrome was also not efficacious.

Twenty patients have been studied in the diagnostic phase of the orthostatic hypotension protocol (Drs. Polinsky, Ebert, Kopin). These patients fall into three clinical categories: (1) predominantly male, middle-aged, and with significant autonomic deficits, as well as evidence for widespread central nervous system disease (Shy-Drager Syndrome); (2) equal sex distribution with wider age range, somewhat less severe autonomic dysfunction and no evidence of central neurological disorder (Idiopathic Orthostatic Hypotension); and, (3) young females with orthostatic tachycardia without evidence of autonomic dysfunction or other neurological signs. A study of the pressor and catecholamine responses to intravenous norepinephrine, angiotensin and tyramine has been completed. In normal subjects, the mean blood pressure is proportional to the log of the plasma norepinephrine level. The change in mean blood pressure is proportional to the log of the dose of angiotensin (ng/min/kg). Using bolus injections of tyramine in normals, no significant change in blood pressure occurs until the dose exceeds 30 μg/kg. Patients with Shy-Drager Syndrome have normal basal plasma norepinephrine levels which do not change appropriately in response to postural change. These patients are also supersensitive to norepinephrine and angiotensin. These data suggest that the distal sympathetic nervous system is relatively intact, but is not appropriately activated by centrally mediated reflexes. Idiopathic orthostatic hypotension patients have low basal plasma norepinephrine levels which change little in response to standing. This group is extremely supersensitive to norepinephrine, angiotensin, and tyramine. The observed responses indicate that the more distal sympathetic nervous system is functioning at a low level despite adequate central impulse traffic -- analgous to ganglionic blockade. Orthostatic tachycardia patients have normal basal levels of plasma norepinephrine which show an exaggerated response to standing and appear to be subsensitive to norepinephrine. The defect in this group might be distal to the sympathetic nerve endings, possibly involving the receptor or effector mechanism.

An examination of the catecholamine response to insulin-induced hypoglycemia has also been completed in the orthostatic hypotension patients. The recovery curve of blood glucose following the lowest level is biphasic. There is an initial phase with a steep slope lasting about 20-30 minutes ("fast phase") followed by a more gradual rise of glucose until normal levels are attained ("slow phase"). The log of the amount of epinephrine released (area under the curve of pg/ml) is proportional to the slope of the "fast phase" and also to the difference in slopes between the fast and slow phases. A number of patients have been identified who show no response of plasma epinephrine during hypoglycemia; these patients did not exhibit a "fast phase" in their glucose recovery curves. Despite an absent epinephrine response, these patients did have intact glucagon, growth hormone, and cortisol responses, suggesting that at least in part these hormonal mechanisms are independent of the epinephrine response.

Significance to Biomedical Research: In recent years, interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. However, there is a beginning literature of clinical neuropharmacological investigations of memory, including work on stages of memory storage pursued recently in our program by Drs. Weingartner and Murphy. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology. Gilles de la Tourette's syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials, and gathering data on cognitive and linguistic function, monoamine metabolism, and neuroendocrine function in this illness, we hope to expand knowledge about its pathophysiology and its possible relationship to other psychiatric illnesses such as schizophrenia. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite.

Proposed Course: The course of this project is related to the development of 2 West as a central nervous system clinical pharmacology service. New diseases may come under study as indicated by available laboratory techniques in the LCS. In the next year we intend to further pursue pharmacological studies of memory function in Altzheimer's disease and in normal controls. We plan to apply two dimensional polyacrilamide gel electrophoresis of plasma and CSF proteins to several dementias. In Gilles de la Tourette's syndrome we intend to pursue neurochemical studies such as those indicated in Project No. Z01 MH 00351-05, and further drug trials. In anorexia nervosa we plan to continue several neuroendocrine protocols as mentioned above, and to study CNS amine metabolism by the probenecid test and by several stable isotope protocols for the study of catecholamine metabolism. The orthostatic hypotension project will progress to several therapeutic drug trials.

Publications:

1. Caine, E.D., Polinsky, R.J., Ebert, M.H., Rapoport, J.L., and Mikkelsen, E.J.: Trial of chlorimipramine and desipramine for Gilles de la Tourette syndrome. Annals of Neurology 5: 305-306, 1979.

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3. Lovenberg, W., Levine, R.A., Robinson, D.S., Ebert, M.H., Williams, A.C., and Calne, D.B.: Hydroxylase cofactor activity in cerebrospinal fluid of normal subjects and patients with Parkinson's disease. Science 204: 624-626, 1979.

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Publications (Continued)

5. Dubois, A., Gross, H.A., Ebert, M.H., and Castell, D.O.: Altered gastric emptying and secretion in primary anorexia nervosa. Gastroenterology, in press.

6. Gross, H.A., Lake, C.R., Ebert, M.H., Ziegler, M.G., and Kopin, I.J.: Catecholamine metabolism in primary anorexia nervosa. J. of Clinical Endocrinology and Metabolism, in press.

7. Nee, L.E., Caine, E.D., Polinsky, R.J., Eldridge, R., and Ebert, M.H.: Gilles de la Tourette Syndrome -- clinical and family study of 50 cases. Annals of Neurology, in press.

8. Davis, G.C., Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M., and Kopin, I.J.: Chronic parkinsonism secondary to intravenous injection of meperidine analogues. J.A.M.A., in press.

9. Williams, A.C., Nutt, J., Lake, C.R., Pfeiffer, R., Teychenne, P.E., Ebert, M.H., and Calne, D.B.P: Actions of bromocriptine in the Shy-Drager and Steele-Richardson-Olszewski syndromes. In Dopaminergic Ergots and Motor Function. Ed.: Calne, D.B. and Fuxe, C.; Raven Press, New York, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00271-10-LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Measurement of Brain Contribution to Norepinephrine Metabolites in Human Urine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Robert L. Sherman	Chemist	LCS NIMH
	Peter Blombery	Guest Worker	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH
	Sanford P. Markey	Pharmacologist	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

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Office of the Chief

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TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gas liquid chromatography-mass spectrometry (GLC-MS), ion exchange chromatography, high pressure liquid chromatography (HPLC) and other separation procedures are used to isolate and measure the specific activities of the norepinephrine metabolites, 3-methoxy, 4-hydroxyphenylglycol (MHPG) and vanillyl mandelic acid (VMA) in the urine of controls and patients with mental and neurologic disorders.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

In humans NE in the sympathetic nervous system and the adrenal medulla is converted to VMA and MHPG. VMA formed is 1 to 2 times as much as the MHPG. Small amounts, 1-5%, of the MHPG is in the free form and the rest is conjugated as the glucuronide or sulfate in approximately equal quantities. In brain, MHPG is the main metabolite of NE, with very small amounts of VMA being produced. Previous work in this and other laboratories suggest that MHPG in the free form is removed from brain directly to the blood.

When ^{14}C dopamine is infused peripherally it does not enter the brain. About 25% of it is converted to ^{14}CNE and its metabolites. Since almost all VMA is formed peripherally its specific activity (ratio of radioactive to non-radioactive VMA) is characteristic of peripheral metabolism. The specific activity of MHPG represents peripheral plus brain metabolism. The difference in specific activities of VMA and MHPG will be a measure of the relative amounts of MHPG produced by brain and periphery. The measurement of absolute quantities of MHPG and VMA formed makes it possible to calculate the amount of MHPG produced by brains of control subjects or patients. The specific activities of the free, glucuronide or sulfate of MHPG may be an indication of the form in which MHPG is transported from brain. Results confirm the earlier finding that MHPG is probably removed from brain as the free compound and conjugated in the periphery.

Radioactive and deuterated standards of VMA and of the three forms of MHPG have been prepared. Separation procedures using ion exchange and high pressure liquid chromatography (HPLC) have been worked out. Final assay is done by gas liquid chromatography-mass spectrometry (GLC-MS) and scintillation counting and is sufficiently sensative to measure levels in human plasma.

In normal subjects 20-40% of urinary MHPG appears to be derived from brain while in patients with orthostatic hypotension believed to be due to peripheral sympathetic nervous system dysfunction there is significantly less MHPG from the peripheral sympathetic nerves but normal amounts from brain. This is being prepared for publication.

Analytical methods have been refined and the urines of a number of controls, patients with anorexia nervosa have been analyzed. Anorexia nervosa patients excrete more NE metabolites after treatment than before. The increase in NE metabolized appears to be divided between central and peripheral sources but more patients must be studies before any firm conclusions can be drawn.

PUBLICATIONS:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00272-08 LCS

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Metabolism, Distribution and Biochemical Effects of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Irwin J. Kopin	Chief, LCS	LCS NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the biochemical effects of drugs in animals and man using mass spectral methodology are being pursued in order to relate pharmacological action to molecular events and clinical efficacy. Work in progress includes synthesis of stable isotope labeled compounds for kinetic studies in man of neurotransmitters, and the effects of various drugs upon the synthesis and release of catecholamines. Drug manipulation of melatonin and 6-hydroxymelatonin levels are being measured to indicate beta-adrenergic receptor activity. Tricyclic antidepressants are being quantitated from patients by mass spectral measurements in order to establish therapeutic and predictive indices. Pharmacokinetic studies of phencyclidine in monkeys are in progress.

PROJECT DESCRIPTION:

Synthetic procedures have been completed for the preparation of deuterated diazomethane, and deuteromethyl analogues of homovanillic acid, 3-methoxy-4-hydroxyphenylglycol (MHPG), and vanillylmandelic acid (VMA). Racemic VMA has been resolved and the natural product (D(-)) has been reduced to D(-) MHPG. This allowed the absolute assignment of configuration to the urinary metabolite of norepinephrine. Studies are in progress to determine if deuterated enantiomers are metabolized at different rates as has been proposed. Resolved deuterated MHPG will be prepared.

The synthesis of di-^{18}O labelled catechol is in progress at the 3-5 g scale with $H_2{}^{18}O$ water supplied by ERDA's Los Alamos facility. Most of the product is to be converted to di-^{18}O labelled dopamine for human studies on peripheral vs. central metabolism of norepinephrine as described in Project Z01 MH 00271-10 LCS.

Two drug related studies utilizing mass spectral methodology developed and collaboratively supported by this laboratory are in progress:

a) Pharmacokinetics of Phencyclidine:
b) Clinical Pharmacology of Antidepressants: Z01 MH 01850-02 CPB

Studies on the levels of melatonin in human plasma using negative ion chemical ionization mass spectrometry (Z01 MH 00274-05 LCS) and the regulation of its synthesis and release from the pineal continue as outlined in Projects Z01 MH 00450-04 (CPB) and Z01 MH 01851-02 (CPB). Effects of various β-adrenergic blocking agents and agonists have been measured in rats in an effort to determine a measure of β-receptor activity.

The difficulty in measuring plasma levels of melatonin led to the study of the major urinary metabolites of melatonin, 6-hydroxymelatonin sulfate and glucuronide. A semi-quantitative assay indicated that approximately 20 ng/ml was present in normal human urine. A quantitative assay employing deuterated standards and enzymatic hydrolysis is currently being developed. The amplitude of pineal secretion can thus be assayed by single 24 hr urine collections for clinical studies of the role of the pineal in various stages of growth, development and disease.

PUBLICATIONS:

Markey, S.P. and Shaw, G.J.: Preparation of highly enriched diazomethane-d_z. J. Org. Chem. 43: 3414-3415, 1978.

Lewy, A.J. and Markey, S.P.: Analysis of melatonin in human plasma by gas chromatography negative chemical ionization mass spectrometry. Science 201: 741-743, 1978.

Markey, S.P., Powers, K., Dubinsky, D., and Kopin, I.J.: General methods for the synthesis of methyl isotope labelled catecholamine metabolites. Preparation of 4-hydroxy-3-methoxy-d_3-(mandelic acid, phenylacetic acid, and phenylethylene glycol). J. Labelled Compds. Radiopharm. (in press), 1979.

Markey, S.P., Colburn, R.W., Kopin, I.J., and Aamodt, R.L.: Distribution and excretion in the rat and monkey of ^{82}Br-bromocriptine. J. Pharmacol. Exp. Ther. (in press), 1979.

Sisak, M., Markey, S.P., Colburn, R.W., and Kopin, I.J.: Identification of 6-hydroxymelatonin in normal human urine by gas chromatography-mass spectrometry. Life Sci. (in press), 1979.

Davis, G.C., Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M., and Kopin, I.J.: Chronic Parkinsonism secondary to intravenous injection of meperidine analogues, JAMA (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00274-05 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Methods of Ionization in Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Robert W. Colburn	Chemist	LCS NIMH
	Masahiro Tetsuo	Visiting Fellow	LCS NIMH
	Wilfried J.W. Mayer	Visiting Fellow	LCS NIMH
	Peter Blombery	Visiting Fellow	LCS NIMH
	Alfred J. Lewy	Staff Psychiatrist	CPB NIMH
	Arthur R. Schultz	Computer Scientist	CSL DCRT
	Leonid Kellner	Visiting Scientist	BEIB

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, NIMH; Computer Systems Laboratory, DCRT, Research Services Branch, NIMH; Biomedical Engineering & Instrumentation Branch

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further work on negative chemical ionization mass spectrometry has shown its general applicability for very high sensitivity analysis of compounds in biological fluids. Following development of a melatonin assay, similar methods have been applied to the analysis of other indolealkylamines (serotonin, 6-hydroxymelatonin) and a variety of biogenic amines and their metabolites (metanephrine, normetanephrine, 3,4 dihydroxyphenylglycol (DHPG), and 3-methoxy-4-hydroxyphenylglycol (MHPG)). Hexafluoroacetone has been shown to produce stable, highly sensitive derivatives for 1,2 disubstituted compounds. A second mass spectrometer has been converted for negative ion detection.

The selected ion recording system developed for use with the LKB 9000 has also been modified to operate with quadrupole mass spectrometers.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Negative ion chemical ionization mass spectrometry has been shown to be uniquely sensitive and suitable for the analysis of appropriately derivatized compounds at the part per trillion (pg/ml) level. Derivatives suitable for electron capture gas chromatography are not necessarily useful for this technique, and thus alternative derivatizing reagents have been tested for this purpose. Hexafluoroacetone can form cyclic ketals or equivalent structures with glycols, amino alcohols, and hydroxyacids. These derivatives have been tested and found to be suitable for human plasma assays of biogenic amine metabolites (as reported in Project Number Z01 MH 00275-02 LCS).

Negative ion chemical ionization has also been applied for the quantitation of urinary 6-hydroxymelatonin, analyzed as the t-butyldimethylsilyl-pentafluoropropionyl derivative.

Preliminary experiments on modified ion sources employing radio frequency filtering are in progress. A collaborative study group at NIH is continuing to focus on the current limitations of analytical mass spectrometers.

The selected ion recording system developed by Mr. Luther Barden with A.R. Schultz of CSL/DCRT has been tested, revised and expanded to accommodate quadrupole as well as magnetic sector mass spectrometers. The system is functional and is in the final stages of project documentation and minor modification.

PUBLICATIONS:

Markey, S.P., Lewy, A.J., and Colburn, R.W.: Quantitative analysis of compounds from biological extracts using negative chemical ionization mass spectrometry. Proc. 26th Ann. Conf. on Mass Spectrometry and Allied Topics, St. Louis, 1978, pp. 393-395.

Markey, S.P., Lewy, A.J., and Colburn, R.W.: Studies in Biogenic Amine Metabolism by Mass Spectrometry. In DeLeenheer, A. (Ed.): *Quantitative Mass Spectrometry in Life Sciences II*, Amsterdam, Elsevier, 1978, pp. 17-37.

Markey, S.P. and Lewy, A.J.: Quantitative analysis of melatonin in human plasma by negative chemical ionization mass spectrometry. In Hertz, H.S. and Chesler, S.N. (Eds.): *Trace Organic Analysis: A New Frontier in Analytical Chemistry*, National Bureau of Standards Special Publication 519, 1979, pp. 309-404.

Barden, L.K., Tate, R.L., Schultz, A.R., Markey, S.P., and Oliver, J.A.: Selected Ion Recording Data System. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, Seattle, 1979, in press.

Blombery, P.A., Markey, S.P., and Kopin, I.J.: Negative EC-CI assays for norepinephrine metabolites. Use of hexafluoroacetone to produce cyclic derivatives. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, Seattle, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00275-02 LCS

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Release and Turnover of Catecholamine Metabolites in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Blombery	Guest Worker	LCS NIMH
Other:	Irwin J. Kopin	Chief, LCS	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Sanford P. Markey	Pharmacologist	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New methods have been developed using negative ion electron capture chemical ionization gas chromatography-mass spectrometry (EC/CI GC-MS) for the assay of the norepinephrine (NE) metabolites, 3-methoxy-4-hydroxy phenylglycol (MHPG), dihydroxyphenylglycol (DHPG), normetanephrine (NMN) and metanephrine (MN). These methods increase the sensitivity of detection of these metabolites 50-100 fold over available electron impact (EI) GC-MS methods permitting the study of NE metabolism in much smaller amounts of biological material than was previously possible.

PROJECT DESCRIPTION:

Assay of catecholamine metabolites in tissue, blood and urine provides information about neurotransmitter release and pathways of metabolism in different tissues. GC-MS methods of assay, in combination with stable isotope labelling of precursors and their metabolites extends the versatility of this technique by providing assessment of rates of metabolism and clearance. Currently available EI GC-MS methods of assay for NE metabolites are limited in sensitivity, and with more sensitive assays, the above techniques could be extended to study NE metabolism in discrete parts of brain and other tissues, as well as allowing assay in very small volumes of plasma.

In this study, suitable derivatives of NE metabolites were investigated for assay by EC/CI GC-MS. The utilization of EC/CI GC-MS depends upon the formation of stable electron-capturing derivatives which provide a high yield of compound-specific anions under CI conditions.

By using hexafluoroacetone to derivatize MHPG, DHPG, NMN and MN, a stable cyclic ketal was formed with good electron-capturing properties. Further reaction with trifluoroacetic anhydride or butylboronic acid formed derivatives which allowed detection of as little as 1-5 pg of the above metabolites in the EC/CI GC-MS mode. This represents a 50-100 fold increase in sensitivity over currently available methods.

Human plasma contains 5 ng/ml free MHPG, 1 ng/ml free DHPG, and 250 pg/ml free NMN and MN. These assays will be utilized to observe changes in plasma metabolite levels during sympathetic activation in normal subjects as well as in patients with orthostatic hypotension, hypertension, depression and other psychiatric disorders.

They will also be used to study the interconversion of the metabolites after administration of deuterated precursors and metabolites. In particular, the conversion of MHPG to vanillyl mandelic acid is being investigated in man, and the incorporation of deuterated methyl groups into MN and the O-methylated NE metabolites is also being studied.

PUBLICATIONS:

Blombery, P.A., Markey, S.P., and Kopin, I.J.: Negative EC-CI assays for norepinephrine metabolites. Use of hexafluoroacetone to produce cyclic derivatives. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, Seattle, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00230-13 LDP

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Intellective Processes in Young Children: Relationship to Cognitive Functioning in Infancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert J. Caron	Research Psychologist	LDP NIMH
Other:	Rose F. Caron	Adjunct Professor Department of Psychology	George Washington University
	Sandra Weiss	Psychologist	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.18	.27

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project was to examine the relations, if any, between information processing in early infancy and intellective functioning in childhood. The Stanford-Binet IQ scores of 4- to 7-year-old children were related to their rates of habituation to regular and scrambled face stimuli at 4 months. Differences in habituation rates in infancy failed to distinguish children on IQ assessments in early childhood. There is some evidence, only for girls, that an association between infant habituation rate and childhood IQ may depend on the meaningfulness of the stimuli used in the habituation procedures.

PHS-6040
(Rev. 10-76)

Project Description:

Although the measurement of mental abilities in infancy has undergone many changes and refinements, and available tests have some diagnostic value in the assessment of early deficits, infant test scores have generally not been predictive of later intellectual functioning. General dissatisfaction with the absence of reliable age-to-age correlations of standard infant tests has led many to question their usefulness as an assessment device for cognitive processing ability. Experimental investigations of infant learning and cognition, on the other hand, continue to provide evidence of robust information-processing capacities in infancy. Investigators are impressed, too, with the range of individual differences in performance and with the possibility that these early differences may prove developmentally stable and predictive of future accomplishment. The purpose of the present project is to determine the extent of the relation, if any, between performance on habituation tasks in early infancy and measures of intellectual ability in childhood.

We had initially hypothesized that, since rapidity of visual habituation may reflect efficiency of information processing, infants who had habituated most steeply to our face stimuli at 4 months of age would exhibit higher IQ scores later in childhood. To provide as favorable a test of this hypothesis as possible, we compared a subsample of 70 infants whose habituation rates were at opposite extremes, i.e., those who had failed to habituate to our least complicated faces (faces with missing features) versus those who had habituated rapidly to our most complex faces. The IQ differences between these groups, while in the predicted direction (114.6 vs. 116.5, respectively), did not approach significance, with males and females showing comparably nonsignificant discrepancies.

We reexamined the data from another perspective. Infants had been habituated to different versions of a schematic face. Some of the stimuli were decidedly facelike or "meaningful;" others were scrambled; others were semifacelike. We hypothesized that whereas rapid habituation to meaningless faces might reflect efficient information-processing, rapid habituation to meaningful faces might index the opposite. At 4 months of age infants are just beginning to internalize the full face schema. An infant who was particularly advanced in this regard might be more likely to notice the facelike stimuli, i.e., to assimilate them to already formed schema. This might cause the infant to fixate these stimuli more intently and thus to retard rate of habituation. Conversely, a child who was late in developing the face schema, might be less likely to perceive schematic faces as meaningful stimuli and would, in general, habituate to them more rapidly than would a more advanced child. If it can be assumed that the earlier an infant develops the natural face schema, the better his information-processing ability, then the correlation between rate of habituation and later IQ should differ depending on the degree of meaningfulness of the habituation stimulus. One would expect the correlation to be negative for our "meaningful" faces, positive for our "meaningless" faces, and somewhere in between for our semi-meaningful faces.

Habituation data collected on 179 4-month-old infants who were subsequently given Stanford-Binet IQ tests between 4 and 7 years of age were reanalyzed.

Findings for girls lend some support to these predictions; the findings for boys tend to be in the opposite direction.

The results of this study will soon be submitted for publication, and the project is now considered terminated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00242-05 LDP

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Development of Sensitivity to the Feeling States of Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
Other:	Carolyn Z. Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	.25	.90

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects on the child of the emotional states of other persons are investigated by naturalistic and experimental procedures. These are studies of children's responses to events of distress (pain, sadness, fear, anger, discomfort). Responding is measured in the child's attending, emotional expression, other overt behavior (e.g., withdrawal, intervention) and verbal expressions. A replication of the longitudinal developmental study (MH-00242-01 to 03) is being carried on with addition of standard cognitive assessments. Thirty-six children (24 in original study, 12 in replication study) are studied over a period of nine months during the second year of life. Daily observational (dictated) reports by mothers and experimentally controlled observations in the home at intervals through the nine months are the data base. The data document progression in acquisition of response patterns indicative of (a) empathic experience, (b) recognition of the separate and different experiences by self and other person, and (c) skills to react overtly in a directed way to the other person's emotions. Children differ in the routes of these acquisitions. Many of the children also develop distinct individualistic response to others' emotions.

Project Description:

The focus of inquiry is the impact on children of emotional events in the immediate environment The concern is primarily with children's responses to incidents in which emotional distress is involved (fear, anger, pain, sadness, discomfort). Emotional dimensions of the environments in which children live have implications for children's functioning. In the routine experience of children, a very large class of stimuli is emotions and feelings of others (e.g., a mother's anger, a father's depression, a sibling's pain, a peer's fear). These emotions can be isolated events or they can be "chronic" in the life of the child. We are interested in how children process these stimuli, and behave in their presence, and how children are affected in a cumulative way.

In the initial study, naturalistic procedures were developed which provided (a) detailed accounts of emotional events occurring in the environment of the family and (b) an accumulation of such accounts over a period of nine consecutive months. Mothers were trained in techniques of observing and detailed reporting of discrete sequences of behaviors. Mothers used a tape recorder to dictate a narrative account of each emotional episode in which another person in the child's environment expressed feelings (anger, fear, sorrow, pain, discomfort).

In addition, at intervals through the 9 months, standard simulated emotions were portrayed by mother and by researcher. These provided standard stimuli and also observation samples for reliability estimates. In the extension of the earlier study, standard cognitive testing is also done in the home at three points in time. Thirty-six children (24 in original study, 12 in replication study) are studied over a period of nine months during the second year of life. Children's responses are assessed in terms of attending, emotional expression, other overt behavior (e.g., withdrawal, intervention), and verbal expressions. The data on developmental transformations in children's responding to emotional distress document progression in acquisition of response patterns indicative of (a) empathic experience, (b) recognition of the separate and different experiences by self and by other person, and (c) skills to react overtly in a directed way to the other person's emotions. Children differ in the routes of these acquisitions. For some, imitative processes are prominent; for some, their own affect plays an important role; for others, the route is predominantly systematic problem-solving behavior. Many of the children develop individualistic, stable patterns of empathic responding to others' emotions in the second year of life.

Analyses are continuing on cognitive-affective interrelations.

Publications:

Yarrow, M. R., & Waxler, C. Z.: Observing interaction: A confrontation with methodology. In Social Interactional Analysis: Methods and Illustrations. Cairns, R. (Ed.), Hillsdale, N.J. Lawrence Erlbaum Assoc. 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00244-04 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
A Behavior Profile for Child Psychopathology and Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)
25 Child guidance clinics and other child mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, MD. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.92	.32	.60

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the research is to develop a standardized behavioral profile for child psychopathology and social competence to be used for research on etiology, treatment effects, epidemiology, and prognosis, as well as for clinical purposes. Parents of disturbed and normal children fill out a behavior checklist describing their child's behavior problems and strengths. Data from the checklists are factor analyzed to identify items that group together within various age and sex groups. The factors are employed as scales of the behavior profile. Empirically obtained distributions of scales on each score provide norms with which the scores of individual children can be compared, and behavior profiles obtained on the same child at different times can be used to assess changes in behavior.

PHS-6040
(Rev. 10-76)

Project Description:

An acute problem in research on the etiology, epidemiology, treatment, and prognosis of child psychopathology is the lack of a standardized and objective system for assessing childhood behavioral disorders. The purpose of the present research is to develop the Child Behavior Profile, an instrument that employs readily obtainable data to portray children's behavioral problems and social competencies in a standardized and objective format.

The data are obtained with the Child Behavior Checklist, which is filled out by parents of children aged 4 to 16 years. Behavior problems (e.g., hyperactivity, disobedience, fears, bedwetting) are scored on three-point scales according to intensity and frequency. Social competence items include reports of participation in sports, hobbies, and organizations, special skills, number of friends and amount of interaction with them, and school performance. The Behavior Checklist is the product of several pilot editions that have been tried out in child guidance clinics and have been progressively revised on the basis of feedback from clinicians, parents, and paraprofessionals.

The data for the Child Behavior Profile have been obtained from Behavior Checklists filled out by parents of children seen in some 25 mental health settings, plus randomly selected parents of normal children. Factor analysis is employed to identify items that tend to occur together in children grouped by age and sex. Several hundred subjects are included in each sample (e.g., boys aged 6 to 11, girls aged 6 to 11, boys aged 12 to 16, etc.)

The groupings of items obtained through factor analysis are used to define the scales of the standardized profile for each age and sex group, and the empirically obtained distributions of scores on each scale provide norms with which the scores of individual children can be compared. The Profile can be used to classify children for purposes of research on etiology, treatment effects, epidemiology, and prognosis, as well as to provide clinicians with a standardized tool for describing and communicating about psychopathology.

Profiles have been completed for boys at ages 4 to 5, 6 to 11, and 12 to 16, and for girls at ages 6 to 11 and 12 to 16, with Profiles for girls aged 4-5 expected to be completed soon. Average one-week test-retest reliability coefficients have been .89 for 6- to 11-year-old boys, .82 for 12- to 16-year-old boys, .88 for 6- to 11-year-old girls, and .90 for 12- to 16-year-old girls. Average interparent agreement coefficients have been .74 for 6- to 11-year-old boys, .79 for 12- to 16-year-old boys, .63 for 6- to 11-year-old girls, and .54 for 12- to 16-year-old girls. Significant stability has also been found in Profiles scored before and after outpatient psychiatric treatment, over a follow-up interval of 18 months. The stability coefficients averaged .63 for 6- to 11-year-old boys, .54 for 12- to 16-year-old boys, .55 for 6- to 11-year-old girls, and .42 for the 12- to 16-year-old girls. Despite decreases in the number of behavior problems reported after treatment, the patterning of behavior remained relatively stable, as reflected in significant Q correlations between the shapes of Profiles obtained at intake and follow-up. The edition of the Profile for 4- and 5-year-old girls is scheduled to be completed during the coming year.

Publications:

Achenbach, T.M. The Child Behavior Profile: I. Boys aged 6-11. J. Consult. Clin. Psychol., 1978, 46, 478-488.

Achenbach, T.M., and Edelbrock, C.S. The Child Behavior Profile: II. Boys aged 12-16 and girls aged 6-11 and 12-16. J. Consult. Clin. Psychol., 1979, 47, 223-233.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00245-03 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Objective Identification of the Hyperkinetic Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Gerald L. Brown	Research Psychiatrist	BPB NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, MD. 20205

TOTAL MANYEARS: .34	PROFESSIONAL: .16	OTHER: .18

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to identify objectively the behavioral patterns associated with findings obtained through intensive medical workups of children referred for hyperactivity. Both parents of a referred child independently complete a behavior checklist from which a standardized behavior profile is derived. Degree of inter-parent agreement is computed and the profiles are analyzed in order to classify children according to types of behavior patterns. Classification of children in this way is then compared with ratings of ward behavior, with the outcomes of the medical work-up, and with the effects of stimulant drugs to determine whether the behavioral typology corresponds to characteristics of the children identified clinically.

PHS-6040
(Rev. 10-76)

Project Description:

The "hyperkinetic syndrome" is today the most widely publicized of childhood disorders. There has been a tremendous increase in the number of children labelled as hyperkinetic and a great deal of controversy over such diverse approaches to treatment as chemotherapy, dietary manipulations, behavior therapy, and laissez faire permissiveness. Etiological hypotheses show a parallel diversity, with minimal cerebral dysfunction, allergies, anxiety, inappropriate reinforcers, constitutional characteristics of temperament, and creative nonconformity all being implicated by one authority or another.

One reason for the diversity of treatment and etiological hypotheses is that there is no objective way to define the behavioral phenotype of hyperkinesis. Since most children are from time to time too active for the tastes of their parents and teachers, and since the terms "hyperactivity" and "hyperkinesis" have gained such wide currency, reports of high activity level alone cannot be regarded as pathognomic. Identifying children who share some common etiological factor with respect to activity level or who would benefit from a particular treatment has thus been made difficult by the excessively broad application of the concept of hyperkinesis.

In order to determine whether there is an identifiable behavioral phenotype corresponding to a clinical syndrome which may include organic anomalies and which may benefit from chemotherapy, collaborative research is being carried out with Dr. G. L. Brown of the NIMH Biological Psychiatry Branch in connection with a study of children referred for hyperactivity (cf. Biological Psychiatry Branch Project Number Z01 MH 0100-04 BP). Both parents of each referred child independently complete the Child Behavior Checklist (described in report on Project Number Z01 MH 00244-03 LDP). An extensive life history is also obtained and the children are admitted to the NIH Clinical Center for several weeks of medical, behavioral, and pharmacological assessment. Without knowledge of the results of these assessments, we are scoring the behavior profile patterns from the Checklist completed by each parent. Interparent reliability is assessed and the separate profiles as well as a composite profile based on the checklists completed by both parents are analyzed to identify particular types of profile patterns. These are being compared with the results of the clinical work-ups to determine whether the types of profile patterns correlate with differential clinical findings and with ratings of ward behavior. Preliminary findings indicate a clearcut behavioral phenotype of hyperactivity and high correlations between parent ratings on our Checklist and one developed by C. Keith Conners for use in evaluating the effectiveness of drug treatments with children.

If further relationships between the behavior profile patterns and clinical findings are confirmed, the profile patterns can be employed to form homogeneous groups for research purposes. As specific etiologies and/or treatments are discovered for these groups, the profile patterns can be employed by clinicians to guide diagnosis and the choice of treatment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00246-04 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

A Follow-up Study of Children Differing in Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)
3 Child Guidance Clinics in New Haven and Waterbury, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.71	.19	.52

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the research are (1) to determine the stability of behavior profiles based on behavior checklists describing disturbed children, and (2) to determine the prognosis for children differing in initial profile patterns. Parents of children for whom Child Behavior Checklists were filled out at intake filled out Checklists describing their children's behavior problems and social competencies following treatment. Behavior profiles based on the follow-up Checklists are being compared with those based on the initial Checklists to determine the stability and differential prognosis for different types of profiles in various age, sex and socioeconomic groups.

Project Description:

Effective utilization of mental health services to children is greatly handicapped by the current lack of knowledge about prognosis for various behavior problems. Although the demand for services to children far exceeds the supply, there is no objective basis for selectively assigning treatment to those who will benefit most, because it is not known which children will actually be helped by treatment, which ones will improve without treatment, and which ones will fail to be helped by current treatment approaches.

A primary obstacle to research on prognosis has been the lack of a standardized and reliable diagnostic-classification system for childhood behavior disorders. As described in the report on Project Number Z01 MH 00244-03 LDP, the investigators have developed a standardized behavior profile for child psychopathology and social competence. It is expected that children having different profile patterns will differ in their prognosis under various conditions of treatment and nontreatment.

To test both the stability and the predictive power of the profile patterns, a follow-up was conducted of 200 children for whom Child Behavior Checklists were filled out in three clinics at intake. Parents of these children were asked to fill out Checklists describing their children's behavior problems and social competencies following termination with the clinics. Statistical analyses are now being conducted to determine whether children who differed in their initial profiles subsequently differ with respect both to their patterns of problems and competencies and to their general adjustment. Preliminary findings indicate statistically significant stability in the patterns of reported problems despite significant declines in the number of problems reported.

Further analyses are expected to show whether initial profile patterns are differentially related to treatment outcome and whether the patterns are useful for determining prognosis under typical clinic procedures. The data will also be compared with outcome data for children seen in other settings and those receiving systematic assignment to particular treatments.

Publications:

Achenbach, T. M. The Child Behavior Profile: An empirically based system for assessing children's behavioral problems and competencies. Int. J. Ment. Health, 1979, 7, 24-42.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00247-04 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relations between Cognitive and Behavioral Patterns in Children with Endocrine Abnormalities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	Roger Johnsonbaugh	Pediatric Endocrinologist	Nat. Naval Medical Center

COOPERATING UNITS (if any)

National Naval Medical Center
Bethesda, Maryland 20014

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.34	.17	.17

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavior checklists and measures of cognitive functioning are being obtained on normal controls and children who have thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive and behavioral adaptation and the relationships between them. The study is designed to test the hypothesis that increases in sex hormone level affect patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex perceptual restructuring. It is also designed to determine whether premature craniosynostonis noted in hyperthyroid childern interferes with cognitive functioning.

Project Description:

One of the most neglected aspects of research on childhood behavior disorders is the interface between cognitive functioning and maladaptive behavior. Research and theory tend to focus on cognitive disabilities as one substantive area and "emotional" problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately interwoven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him or her. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which children make use of their cognitive capacity.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostonis in these children, and it is important to determine whether cognitive and behavioral patterns are affected by this condition. The children and their sibling controls are recruited by Dr. Roger Johnsonbaugh through his pediatric endocrinology service at the Naval Medical Center.

Our measures of cognitive functioning include the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, a measure of speed of color naming, and a measure of speed of object naming. Differences between perceptual restructuring measures (WISC Block Design, Embedded Figures Test) and automatization measures (color naming, object naming) will be compared at two testings to determine whether changes in hormone levels after medication cause changes in cognitive patterning that do not occur in normal, unmedicated control subjects.

Preliminary findings indicate some differences in cognitive patterning between children with thyroid abnormalities and their normal controls, but not enough subjects have yet been tested to determine the generality of these differences or to draw conclusions about the effects of sex hormones.

Publications:

Edelbrock, C. and Sugawara, A. I.: Acquisition of sex-typed preferences in preschool-aged children. Dev. Psychol. 1978, 14, 614-623.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00250-04 LDP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Information Processing in Premature and Full-term Infants at the Perinatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Research Psychologist	LDP NIMH
OTHER:	Blanche S. Jacobs	Research Psychologist	LDP NIMH
	Milton W. Werthmann, Jr.	Chairman, Dept. of Perinatal & Neonatal Pediatrics	Washington Hospital Center, Wash., D.C.

COOPERATING UNITS (if any)

Washington Hospital Center, Washington, D. C.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.34	.77	.57

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study sensory function and temperament were compared in infants varying in gestational and medical status at birth. The infants who were studied included low medical risk preterms at their expected date of delivery, healthy full-term neonates, and post-term neonates. Sensory function was evaluated by measures of response to novelty, level of responsiveness, and response decrement collected when the infants were responding to tactile, auditory, and visual stimulation. Temperament was assessed on the basis of the spontaneous behaviors of the babies, their responsiveness to soothing when they cried, and their responsiveness during a neurological examination. Temperament was described in terms of state organization, irritability, soothability, and activity level. Preterms were different from full-terms on auditory, visual and temperamental measures. Post-term infants differed from full-terms on some temperamental measures.

PHS-6040
(Rev. 10-76)

Project Description

Most pregnancies last between 38 and 42 weeks. Some, however, terminate much earlier or later. Medical technology has made it possible for infants born as early as at 28 weeks or as late as at 50 weeks of gestation to survive. This is certainly an impressive medical accomplishment; yet, the psychological outcomes of too short or too long periods of gestation are not fully understood. It is not obvious that the central nervous system of a preterm organism is ready to benefit from extrauterine stimulation. Nor is it obvious that a post-term organism has not suffered because of its not being exposed to an extrauterine environment at a possibly critical period for some aspects of development. Birth not at term is often but not always associated with medical complications. This raises the question about the role of illness in early development. It is possible that a newborn who spends much energy in a struggle for biological survival has very little energy left for psychological development.

Evidence from late infancy and childhood indicates that children who were not born at term are at a greater than average risk for intellective and behavioral deficits. In this study we explore some precursors of intellective and behavioral deficits in early infancy -- in the days after birth or at the expected date of birth (in the case of preterms). Knowledge of specific weaknesses and strengths at this early stage should make it possible to rationally plan early intervention programs to stimulate those aspects of development which show deficits due to birth not at term.

The psychological functions which were studied are of two classes: Sensory processing as an index of the degree of cortical maturity and integrity and temperament. Sensory processing was assessed in the tactile, auditory and visual modalities. In the examination for tactile processing, the infant's left cheek was stroked repeatedly by one of two non-noxious stimuli (a nylon filament applied in two directions) for 2-second periods, with a random interstimulus interval. When the infant reached a pre-determined response decrement criterion, the other stimulus was applied until the same criterion was reached again. Behavioral data were collected providing measures of response to novelty, level of responsiveness and response decrement. In the auditory information processing examination, the infant was presented with one of two tones (500cps; 1500cps at 80db). The procedures and measures were equivalent to those described for the tactile modality. In the visual processing examination, each of two stimuli (revolving, lighted, differently colored plexiglass boxes) were presented repeatedly for 30-second periods starting with the infant's first look at the stimulus. Visual attention data were collected.

Temperaments of children are believed to influence parent-child relationships, and these relationships in turn are believed to influence the social and cognitive development of children. We were interested in finding out whether non-term infants were temperamentally different than full-term infants. Such differences could feed into abnormal infant-caregiver interaction and might lead in its extreme cases to child abuse.

Temperament was assessed by the state of arousal and behaviors of the infant when he/she was free of discomfort, when he/she was crying and responding to soothing, and during a neurological examination. During 18 minutes the infant was observed for periods of 5 seconds, followed by 10-second periods in which the observer coded the infant's behaviors. Infant behaviors which were coded included state of arousal, body, limb, and facial movements as well as looking activity and vocalizations. If the infant cried, an experimenter intervened in a progressive manner in an attempt to sooth the infant. A brief neurological exam tested for the neck muscle control, arm and leg recoil, arm and leg traction, head raising, arm release, the Galant reflex, the sucking ability, the rooting response, foot withdrawal and the moro reflex. At five specified times during the exam, the arousal state of the infant was coded. Twenty-three full-term, 45 preterm, and 9 post-term infants were studied.

Full-term and pre-term infants were equally responsive to the tactile stimulation. Full-term infants were somewhat ($p < .10$) quicker to respond to the initiation of auditory stimulation and responded more vigorously on the first few trials ($p < .05$). Full-terms were also quicker to respond to the initial presentation of the visual stimulus ($p < .05$) and took less time to reach the visual response decrement criterion ($p < .05$). Post-term subjects were not deficient relative to the full-term subjects in their tactile, auditory or visual responsiveness.

Temperament data show that the full term subjects were of "easier" temperamental dispositions. They had less labile state organization ($p < .001$), were fussing and crying less ($p < .02$, $p < .04$), and were less active ($p < .02$) throughout the observations of spontaneous behaviors. They were also less irritable and easier to sooth during the neurological exam ($p < .01$, $p < .01$). Full-terms were also temperamentally easier than post terms. They had a less labile state organization ($p < .02$), and they were less irritated by tactile stimulation ($p < .01$) and by the neurological exam ($p < .04$). However, once upset, they were more difficult to sooth than the post-term subjects ($p < .04$). Our results show that preterms, and to a lesser extent post-terms, present themselves as temperamentally more difficult than full-term infants.

Data analysis is currently in progress, and additional articles are being written.

Publications:

Friedman, S. L., Jacobs, B. S., and Werthmann, M. W. Tactile, auditory, and visual processing in pre-term, full-term, and post-term infants at the neonatal period. In Friedman, S. L. and Sigman, M. (Eds.): Pre-term and Post-term Birth: Relevance to Optimal Psychological Development. San Francisco, Academic Press, 1980, In Press.

Brackbill, Y. and Friedman, S. L. Ethics of intensive care and research with pre-term infants. In Friedman, S. L. and Sigman, M. (Eds.): Pre-term and Post-term Birth: Relevance to Optimal Psychological Development. San Francisco, Academic Press, 1980. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00253-04 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Behavioral Studies of Seriously Ill Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth J. Susman	Guest Worker	LDP NIMH
Other:	Albert R. Hollenbeck	Guest Worker	LDP NIMH
	Ellen D. Nannis	Social Science Analyst	LDP NIMH
	Barbara E. Strope	Social Science Analyst	LDP NIMH
	Stephen P. Hersh	Assistant Director for Children and Youth	NIMH
	Arthur S. Levine	Chief	POB NCI
	Philip A. Pizzo	Senior Investigator	POB NCI

COOPERATING UNITS (if any)
Pediatric Oncology Branch, National Cancer Institute

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.87	.41	.46

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical well-being is an assumed, though generally unmeasured, condition in studies of the child's psychological development and behavior. There is little developmental inquiry concerning the effects of absence or loss of physical well-being on child development, despite the reality that acute or chronic ill health from disease or accident is, to a great or small degree, part of the life of every child. This project is concerned with the psychological and social impact of serious illness (cancer) on children and those in their social network (namely their families and caregivers). Areas of research include coping abilities, the influence of the social system of the child, and the mutual influences of the stressed children and those in their social network. Observations, interviews, and parent and nurse ratings were used to investigate these phenomena.

Project Description:

The purpose of this project was to assess the psychological impact of a serious illness (cancer) on children, with particular emphasis on the child's understanding of the illness, the child's subjective experiences associated with illness, and the child's social behavior with significant others while hospitalized.

The research participants were patients and their families of the Pediatric Oncology Branch of the National Cancer Institute. The sample consists of children, 18 months to 21 years, with metastatic solid tumors and lymphomas that had failed to respond to conventional therapeutic regimens. These children received intensive chemotherapy which is very toxic and can lead to life-threatening complications. Research participants were housed in either Laminar Air Flow units (a protective germ-free environment) or a general pediatric oncology unit for a period of three to seven weeks. In the Laminar Flow units, children are deprived of important social contacts--parent, siblings, and peers--as well as the normal sources of stimulation in the environment.

The research was designed to explore the child's and parent's conception of the illness--its cause, course, and prognosis--and how these conceptions change over time; the relation between the child's conceptions of the illness and emotional states and social behavior during hospitalization; and the role parents and medical personnel play in providing comfort and support to the hospitalized child.

For the older children (*n* = 19), multiple methods were used for data collection: systematic observation, interviews, and parent and nurse ratings. Children were interviewed at the beginning and end of chemotherapy. Observations and ratings were done daily throughout hospitalization. For the younger children (*n* = 4), observations were done twice a day, four times a week.

Structured interviews used to assess children and parents' conceptions of a life-threatening illness were analyzed for generational differences, age differences, and longitudinal change. Mother and child's conceptions of a life-threatening illness were significantly related on general knowledge of illness and affective variables assessing hopefulness for the future, the degree of reality involved in planning for the future, and the degree to which the child plans to become involved in activities of daily living after leaving the hospital. Cognitive understanding of the illness is best predicted from knowing the age of the child, while the child's affect associated with an illness is best predicted from knowing the mother's affect.

A child or parent's perception of control over the illness and treatment emerged as an important aspect of coping with illness. Mothers and children high on perceived control are more optimistic, more oriented toward future goals, and more oriented toward living rather than toward the possible negative outcomes of the illness, than mothers and children low on perceived control. Thus, although children with cancer and their parents have little objective control over the disease, a subjective impression of control has important implications for psychological adjustment to the illness.

The behavior of 23 children was observed while they were confined to Laminar Air Flow Rooms or to a regular pediatric oncology unit. The data were analyzed for behavior change within individual subjects: (a) to determine whether behavior changes were related to physiological changes associated with the effect of chemotherapy, and (b) to identify trends in behavior that occurred with regularity across all children.

One side effect of chemotherapy is a reduction in white blood cells. Thus, as a marker of physiological integrity, the period of observation was divided into blocks of time based on white cell counts greater than 500 (Period 1), less than 500 (Period 2), and greater than 500 (Period 3). For each child, a single subject design multiple regression analysis was performed on six behavior categories and the blocking variable, white blood count. The behaviors included: Play, Sleep, Vocal, Passive, Physical, and Protest.

Findings indicate that the pattern of behavior change over time is not consistently related to change in white blood cells. Although there was a tendency for Play to decrease and Sleep to increase with decreases in white cell count, there is also evidence that the increases or decreases sometimes preceded the actual change in cell count. Also, once a behavior began to change in a specific direction, it continued to change in that direction regardless of the corresponding physiological changes. The finding that behavior did not approach baseline levels as physiological integrity was restored leads to the speculation that other factors mediate the impact of physiological variables. Results indicate the importance of caregivers as moderators of the possible detrimental effect of long-term institutionalization on development.

Further reports are being prepared for research journals.

Publications:

Levine, A. S., Artiss, K. L., and Susman, E. J. The Doctor-Patient and Nurse-Patient Relation: An Exploration of the Roots of Anxiety. *Proceedings of the American Society National Conference on the Care of the Child With Cancer*, 1978.

Pizzo, P. A., Levine, A. S., Marshall, D., Susman, E. J., and Robichaud, K. Past, Present, and Future Studies of Protected Environment, Regimen and Cancer Treatment. *J. of Environmental Science*, in press.

Susman, E. J., Hollenbeck, A. R., Hersh, S. P., Levine, A. S., and Pizzo, P. A. Separation-Deprivation and the Child with Cancer: A Re-evaluation. In Kellerman, J. (Ed.), *Psychosocial Aspects of Childhood Cancer*. Springfield, Ill., Charles C. Thomas, in press.

Susman, E. J., Hollenbeck, A. H., Nannis, E. D., and Strope, B. E. A Developmental Perspective on Psychosocial Aspects of Childhood Cancer. In Schulman, J. L. and Kupst, J. J. (Eds.): *The Child With Cancer - Clinical Approaches to Psychosocial Care - Research in Psychosocial Aspects*. Springfield, Ill., Charles C. Thomas, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00255-03 LDP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Maternal Imprisonment: Psychological Effects on Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Velma LaPoint	Staff Fellow	LDP	NIMH
OTHER:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Barbara Strope	Social Science Analyst	LDP	NIMH
	S. Lawson Sebris	Psychologist	LDP	NIMH

COOPERATING UNITS (if any)
Maryland Division of Corrections, Department of Public Safety, Baltimore, Md.
Institute of Law Enforcement and Criminal Justice, Law Enforcement Assistance Administration (LEAS), Department of Justice, Washington, D.C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.76	1.05	1.71

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The lack and/or loss of stable and supportive parenting places children at risk for the development of mental health problems. Psychological risk for the child is compounded when the reasons for inadequate parenting are themselves indicative of psychological problems of the parents. The purpose of the study is to identify psychological consequences for these children whose mothers are imprisoned. The relations between mother and child, attitudes toward self, mood and emotional states, and the child's interpersonal relations with peers and adult authority figures are studied. Research participants are 40 incarcerated mothers and their children, and equal numbers of mothers and children from single-parented families in the community. The groups are comparable racially (Black) and socio-economically. Data are obtained in four settings: mother groups, caretaker settings, mother-child interaction with their children, and experimental child groups. Secondary data are obtained from school records.

Project Description:

The loss of stable and supportive parenting place children at risk for mental health problems. Risk for the child is compounded when the reasons for inadequate parenting are themselves indicative of psychological problems of the parents. Children whose mothers are imprisoned constitute such a group at risk. An estimated 157,000 American children, in any one year, are in this group. The number of children who grow up with this experience are many multiples of this number.

The purpose of this study is to identify psychological consequences for children of mothers' imprisonment. Four aspects of consequences are examined: the relationship between mother and child; the child's moods, emotions, and attitudes toward self; the child's interpersonal relationships with peers and adult authority figures; and the child's abilities to cope with daily demands of living.

The subjects are 40 imprisoned mothers at the Maryland Correctional Institution of Women, Jessup, Maryland and their children. Children presently live with substitute caregivers, and are between 5 and 12 years old. Two comparison groups of single-parented families, in the community matched on race and socio-economic status but without maternal histories of institutionalization, are also studied. One of these groups is made up of 30 families without previous maternal involvement with law enforcement authorities, and the other is composed of 30 mothers currently on probation. Data are obtained in four settings: mother discussion groups, caregiver and child in the home, mother and child groups during visits to the prison, and experimentally-created child groups in community settings. A secondary data source is school records of children's academic and behavioral performance.

Data are obtained on mothers' roles and attitudes toward their children, and attitudes toward themselves as mothers. Mothers are observed in small group discussions; they are observed also with their children in small groups and, each mother each mother is seen with her child in standard dyadic situations.

The primary data on the child are observations of interactions with mother, and observations of behavior in experimental child groups. Assessments include relationships between child and mother, emotional state, attitude toward self, attitudes toward authority, and problems in coping with day-today demands. Data collection is ongoing.

Findings

Analysis of self reported data from imprisoned mothers revealed a variety of maternal behaviors and mother-child relations prior to and during imprisonment: The majority of mothers had had regular involvement in the lives of their children prior to imprisonment. However, most were single mothers, which would suggest unique handicaps in the mother-child relationships. Mothers often had child care support from their extended families, typically their own parents.

Current mother-child relationships range from warm and responsible to strained and tense. Some mothers follow in detail what is occurring in their children's lives; some mothers know little about what their children are doing or experiencing.

Mothers indicated that children were knowledgeable of the mother's offense, either hearing directly from mother or family or witnessing the actual event. The latter included observations of the deviant behavior and/or police apprehension of mother. Children sometimes accompanied mothers to court as well as visited mothers in local detention centers and in prison. The child was directly involved in many ways.

Many mothers expressed guilt feelings and concern about how imprisonment would influence the child. The mothers had great difficulty in explaining their offense to their children. They were embarrassed or afraid to disclose the truth. Mothers and family members told the children to conceal their mother's status. This appears to be protective of child as well as mother. Mothers reported children being teased by peers about the mother's status. Thus, children are experiencing social punishment while mothers undergo imprisonment.

Data collection is ongoing and further analyses, including study of children's responses, are underway.

Findings from this study of mothers' imprisonment will provide information about children's and family coping with the stress. The findings also have implications regarding policies for imprisoned mothers and their children. A wide range of correctional policies is being considered, yet there is little knowledge about the nature of the mother-child relationship of imprisoned mothers or about the effects of mother's imprisonment on children.

Publications:

LaPoint, V. Mothers Inside, Children Outside: Some Issues Surrounding Imprisoned Mothers and Their Children. Proceedings of the 107th Annual Congress of Corrections of the American Correctional Association, Maryland. American Correctional Association, 1978, pp. 274-284.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00256-04 LDP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Expressions of Affect: Children's Age and Sex-role Expectations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karabelle A. L. Pizzigati	Guest Worker	LDP NIMH
OTHER:	Gale E. Inoff	Psychologist	LDP NIMH
	Charles F. Halverson, Jr.	Professor	Dept. of Home Economics, Univ. of Georgia, Athens

COOPERATING UNITS (if any)

Green Acres School, Rockville, Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.51	.46	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children's judgments with regard to sex-role-related personality traits were investigated. Children, ages 5-13 years, viewed videotaped skits portraying traits stereotypically associated with one sex or the other (e.g. gentleness, stoicism). The children evaluated themselves, considered peers in terms of the traits portrayed, and assessed the social desirability of the traits. The findings indicated only modest concordance between the children's perceptions of themselves (as well as their peers) and the stereotypes. No age patterns were found consistently across traits. The self ratings were related to ratings of social desirability of the traits, especially at the youngest age period. It seems that, when cued in by the task or instructions, children can accurately report the content of stereotypes and use them "appropriately." When not cued in to stereotyping, however, they may or may not use stereotypes to organize their information processing. Investigators need further to identify the characteristics of situations which elicit the use of stereotypes.

Project Description:

The purpose of this project was to investigate the development of children's perceptions of sex-role-related personality traits. The importance of the gender dimension in children's perceptions of themselves and their peers was studied. The sample consisted of 150 middle and upper-middle class elementary school boys and girls attending a private school. Each participant individually viewed 10 videotapes portraying the following traits: gentleness, fickleness, emotional expressiveness, dependency, nurturance, stoicism, messiness, assertiveness, independence, and boastfulness. Evidence from the literature and from pilot data suggests that these traits relate to sex-role attitudes. For each trait, the children were asked to describe the portrayal, rate themselves on the trait portrayed, nominate two peers who exhibit the trait, and evaluate the social desirability of the trait.

When looking for evidence of sex role stereotypes, one would expect a clustering of traits that represent each sex role. In the children's self-ratings, although the correlations which replicated across age groups were almost always masculine traits correlating with masculine traits or feminine traits correlating with feminine traits, the traits did not fall into sex role clusters. There was no evidence that masculinity and femininity are anchors of a bipolar dimension. A recent view is that masculinity and femininity are independent dimensions and that androgyny is a possible (as well as an adaptive) orientation; we, however, did not find evidence for dimensions.

From traditional stereotypes one might predict that, with age, boys would become more masculine and less feminine and that girls would become more feminine and less masculine. Cross-sectional analyses for age differences between groups lent meager support for this hypothesis. There was no consistent pattern across traits. Only when the requirements for a sex role were made less stringent did we find significant age differences--boys decreasing in self-ascribed femininity with age. No masculine sex role was found even when the requirements were eased.

Although clustering of traits and age differences are not necessary to infer that the self ratings are in accordance with a stereotype, one would expect differences between the sexes. There were more sex differences in self ratings than one would expect by chance, but the results were not compelling. Almost all of these sex differences were on the feminine traits. This finding is compatible with results of other studies concerning avoidance of sex-inappropriate activities and differential socialization of such behavior. Since girls more readily engage in cross-sex behavior, it might be expected that sex differences would be attenuated for the masculine traits.

Self ratings also were analyzed in relation to ratings of social desirability of the traits. The self ratings were correlated with social desirability, especially at the youngest age period.

When the children named peers whom they felt exhibited each trait, there was some concordance with the stereotype, but the most likely response was to name same-sex peers, regardless of the trait.

In children's self perceptions and peer nominations, sex role stereotyping was not readily apparent. However, when children were asked whether each trait was "for boys" or "for girls," there was considerable sex typing in line with the larger society's stereotype. It appears that, when told to do so, children can accurately report the content of stereotypes and use them "appropriately." When not cued in by task or instructional sets, they may or may not use stereotypes to organize their information processing. The task presented to the children seem to be an instance in which most of the children did not spontaneously use sex role stereotypes.

A manuscript of this study has been submitted for publication.

Publications:

Inoff, G. E., Halverson, C. F., Allen, M. G., and Cohen, D. J. Pathways between constitution and competence during the first five years of life. _Dev. Psychobiol._ In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00257-03 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	David G. Poplack	Senior Investigator	POB NCI
	Van S. Hubbard	Clinical Associate	NIAMDD
	Stuart Siegel	Chief, Div. of Hematology-Oncology	Los Angeles Children's Hospital, Los Angeles, California
	Jonathan Kellerman	Pediatric Psychologist	Los Angeles Children's Hospital, Los Angeles, California

COOPERATING UNITS (if any)
NCI
NIAMDD
Los Angeles Children's Hospital

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.43	.42

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of children with leukemia are investigated. One study compared a group of leukemic children in remission with a matched group of sibs on measures from a battery of psychological tests (Wechsler, Bender-Gestalt and Graham-Kendall). These groups are followed-up 12-15 months after the initial assessment. A sample of leukemia patients who have not received CNS treatment and their sibs and a sample of cystic fibrosis patients and their sibs are also being assessed using these procedures to control for effects other than CNS treatment. A second study deals with another sample studied prospectively. In this instance children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are re-evaluated one year later to determine if any possible brain changes brought on by the CNS treatment have resulted in learning disabilities and diminished intellectual functioning. The healthy siblings of the leukemics receiving CNS treatment, who are being studied retrospectively, will be used as a control group to check testing effects.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The chance of survival from childhood leukemia has increased greatly over the past 10 years because of the utilization of new treatment procedures. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents.

The purpose of the proposed research is to determine if CNS treatment has a deleterious effects on intellectual functioning and to evaluate the nature and extent of these effects. Two studies are in progress. One study involves evaluation of intellectual functioning only after CNS treatment (post-treatment study); in the other study intellectual performance prior to CNS treatment is compared to performance subsequent to treatment.

Because of the age range of our subjects,the three Wechsler Intelligence Tests are used: WPPSI (ages 4-6), WISC-R (ages 6-16), WAIS (17 years of age and over).

Study I. Post-treatment study

The sample consists of 34 children who have been in remission for 2 to 5½ years from acute lymphocytic leukemia (ALL), and a sibling control group. The mean age of the children at the time of assessment was 11 years with a range of 4 to 23. All children were administered a Wechsler Intelligence Test, the Graham-Kendall and the Bender-Gestalt.

For most comparisons the sibling group performed at a significantly higher level of functioning than the patients. The mean Full Scale IQ of 99 for patients and 112 for siblings indicates that the patients are operating at an average and the siblings at a bright average level of intellectual functioning.

A hypothesis which we investigated was that although the damage from the CNS treatment might not have greatly diminished mental abilities already present at the time of treatment, it might have interfered with the acquisition of new skills and the mastery of higher levels of mental functioning that ordinarily would be consistent with continuing development. Thus, as the individual aged he or she would fall further behind developmentally. Since the time interval between the time of CNS treatment to the time of the psychological evaluation varied among patients, analyses were done to determine whether time since treatment was associated with the degree of intellectual deficit. The patient's sibling's score was used as an estimate of the patient's potential level of functioning. The patient sample was dichotomized on length of time between treatment and time of testing and the two resulting groups were compared on the difference scores between each patient's score and his/her sibling's score. The longer interval group had the greater difference scores although none of these findings were statistically significant.

To test the hypothesis that younger children are more impaired by CNS treatment, the patients in our sample were dichotomized into an older and a younger group (based on their age at the time of treatment), and difference scores between them and their siblings were derived in the same manner as described previously. The younger S's had significantly greater decrements in Full Scale and Verbal Scale IQ scores than the older group of patients. A non-significant trend in the same direction held for the performance scale IQ scores.

The age at treatment and the time interval since treatment were combined into one variable in order to maximize the variance contributed by these two theoretically related variables. (Age at the time of testing divided by the time since testing, yielded a score on the total percent of life lived since treatment). The patients were then dichotomized into those who lived either a greater or shorter percentage of their lives between treatment and testing; these two groups were compared as to the degree to which their scores differed from those of their siblings. The findings parallel those cited above, but the combined variable produced more highly significant differences than did the two separate variables.

To test the hypothesis that intellectual skills of brighter individuals tend to be more affected than those of average individuals by the diffuse brain damage likely to be produced by the CNS treatment, each patient was given an estimated pre-treatment IQ based on the IQ of the sibling. The sample was dichotomized into high and average IQ. Difference scores were then computed between the patient and the sibling. The difference scores were greater for the patients with brighter siblings and most of the comparisons between groups were statistically significant. (There is very little evidence from a comparison sample that regression contributed in any major way to the apparent greater drop in IQ of patients with brighter siblings). There was also a significant difference between patient and sibling performance on the Bender-Gestalt test ($t = 1.79$, $p < .1$) although no significant difference on the Graham-Kendall test. Twenty-six patients and their siblings were retested 12-15 months after the initial testing. Patients who had relapsed were not included in the retest sample because they received additional treatment. No significant differences were found on any of the tests between the patients' or the siblings' initial and follow-up testing.

A group of 22 children with cystic fibrosis who were not being treated for exacerbation of their symptoms and their healthy siblings was used to control for the psychological, social, and medical effects of chronic illness on mental abilities. In 8 of the 11 tests, patients scored higher than did their healthy siblings although not significantly so, when results were analyzed using a two-tailed t test. However, using a nonparametric statistic, Mann-Whitney U test, CF patients did score significantly higher ($p < .1$) on the overall performance scores.

Thirteen leukemia patients who were treated for their leukemia without the use of CNS treatment and their siblings served as a third control group.

There were no significant differences between these patients and siblings on any of the measures.

Study II. Prospective study

A sample of 60 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, are being administered the same psychological evaluation described in the Post-treatment study. This assessment is made before CNS treatment and one year after treatment. This interval of one year should be sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The sibling group of leukemic patients receiving CNS treatment who were a part of the Post-treatment study will be used as a control group for this study to partial out any practice effects that may contribute to the follow-up IQ scores among the patients.

Half of this sample will be between 3½ and 6 years of age and half will be between 6 and 16 years of age. This age distribution will provide a good basis for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit that may occur. Initial testing has been done in 26 cases and the one year follow-up in 7 cases.

Publications:

Moss, H. A., and Nannis, E. D. Psychological Effects of Central Nervous System (CNS) Treatment of Children with Acute Lymphocytic leukemia (ALL). In Kellerman J. (Ed.): *Psychosocial Aspects of Childhood Cancer*. Springfield, Charles C. Thomas, Publisher. In press.

Appendix to Project Number Z01 MH 00257-03

Contract # 263-MD-900466 for $1320 has been used to reimburse testers, $40 per case. The tests given are the WISC. The work involved is the equivalent of 1/20 man years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00259-03 LDP

PERIOD COVERED

October 1, 1979 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relations between Parent, Teacher, and Clinician Reports of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	Faith Dickerson	Clinical Psychologist	Woodburn Center for Community Mental Health, Annandale Virginia

COOPERATING UNITS (if any)

Woodburn Center for Community Mental Health, Annandale, Va.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.34	.13	.31

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relationships between parent, teacher, and clinician reports of the behavioral problems and competencies of children referred for outpatient mental health services; (2) to determine the relationship between children's presenting behavioral patterns as reported by these sources and the outcome of their clinic contacts. At intake, and again following termination with the clinic, parents and a clinician filled out the Child Behavior Checklist, while the child's teacher filled out a Teacher Report form. Child Behavior Profiles scored from these data have been compared to identify agreements and disagreements between parents, teachers, and the clinician. The relationship of outcome to intake Profile patterns, demographic variables, and other characteristics of the children will also be assessed in order to identify predictors of poor outcome. If children who typically have poor outcomes can be identified, new treatment strategies may be developed to help them.

Project Description:

Little is known about the relationships between client characteristics and the effectiveness of existing treatments for child psychopathology. One of the purposes of the Child Behavior Profile (Project No. Z01 MH 00244-04 LDP) is to differentiate among children who differ in their responses to various treatment modalities. This study is designed to determine the degree of agreement and the nature of disagreements between Profiles scored from Child Behavior Checklists completed by children's parents, their teachers, and a clinician who did an intake and follow-up evaluation but was not involved in treatment. It is also designed to determine whether classification of children according to Profile patterns derived from reports by each type of informant separately and/or in composite is predictive of outcome in response to the child mental health services offered by a community mental health center.

In addition to the Behavior Profiles, the intake clinician obtained data on family composition, demographic characteristics, previous mental health contacts, degree of parent motivation for help, and the problems considered most serious by the parents. Six months after intake into the clinic, the intake clinician contacted the family to obtain Behavior Checklists describing the child's current status, plus data on changes in the family, the family's reactions to the services they have received, and possible needs for further services.

If children who have poor outcomes can be discriminated from those who have good outcomes on the basis of intake data, then new treatment strategies can be developed for helping children who are not likely to benefit from mental health services as currently offered. Initial analyses show correlations averaging about .80 between Child Behavior Profile scales scored from Checklists filled out by the clinician and the children's mothers at intake and about .75 at follow-up; correlations averaging about .41 between the clinician's ratings and teachers' ratings at intake and about .51 at follow-up; and correlations averaging about .28 between teachers' ratings and mothers' ratings at intake and negligible correlations at follow-up. Further analyses will be made to identify the best predictors of outcome as judged by parents, teachers, and the clinician.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00260-03 LDP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Intellective Development in Normal and Deviant Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert J. Caron, Ph.D.	Research Psychologist	LDP NIMH
	Rose F. Caron, Ph.D.	Adjunct Professor	George Washington University
	Anita Sostek, Ph.D.	Pediatric Psychologist	Georgetown Univ. Hospital
	Gordon Avery, M.D., Ph.D.	Director, Division of Neonatal Medicine	Children's Hosp.
	Sandra Weiss	Psychologist	LDP NIMH

COOPERATING UNITS (if any)

George Washington University
Georgetown University Hospital
Children's Hospital

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to lay the basis for the development of a behavioral assay of intellective deficit in early infancy. Normal and high-risk infants are compared in terms of their ability to code relational features of stimuli under the assumption that the processing of patterned information is fundamental to emerging intelligence. Habituation-recovery procedures are being used to assess sensitivity to invariant spatial, temporal and social relationships between 52 and 78 weeks conceptional age. Overall, the findings indicate that preterm infants are much less sensitive to the configuration of stimulus elements than full term infants.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of the project is to develop an assay of intellective functioning early in life. The research focuses specifically on the infant's ability to abstract relational information. The guiding assumption is that vulnerability to future mental deficit will manifest itself early in life as a difficulty in abstracting invariant spatial, temporal, and physico-structural relationships from the environment. The research purpose is to determine when, and under what conditions, normal infants are able to abstact patterned information, and to compare the ability of premature and healthy infants in this regard.

The first study was concerned with the capacity of 12-week-olds to respond to the configuration of the human face and to ignore facial details such as the shape of eyes and nose. Twenty-two healthy, full term infants and 20 preterm infants were habituated visually to four drawings of a schematic face in which the eyes and nose differed across stimuli (e.g., eyes and nose of circles, crosses, triangles, diamonds). Following habituation, they were shown two new faces, first in the familiar configuration and then in a scrambled configuration. If the infants had abstracted the invariant face pattern during habituation, they would be expected to generalize habituation (continue to look briefly) at the new instances of the face and to dishabituate (increase fixation) to the novel configuration. Term infants performed precisely in this manner. By contrast, preterm infants looked no more at the novel (scrambled) patterns than at the face patterns. These results are impressive, since no attempt was made to differentiate between high and low risk among preterm infants, so that this group undoubtedly included cases of marginal jeopardy.

Another group of 12-week-old term infants was habituated to a single exemplar of the face pattern and then shown in the same four test figures. It was hypothesized that the face configuration might not become as salient relative to the face details in the single-exemplar condition as in the previous four-exemplar situation. Contrary to prediction, this group yielded almost the identical pattern of test results.

In the second study, the ability of 18-week old infants to respond to an invariant spatial relation (above-below) despite variation in the shape of the component figures was examined. Twenty term and 19 preterm infants were habituated to four exemplars of a "little-above-big" relation, whereupon they were shown two new shapes in the familiar pattern and in a novel pattern. Again, the full term infants yielded test results indicating that they had encoded the invariant "above" configuration during habituation, in contrast to the preterms.

In the third study the ability of older infants (21 weeks) to detect a quite subtle relational feature (same-different) was investigated. Twenty-four term and 12 preterm infants were habituated to four exemplars of the "same" relation using pairs of cartoon faces (AA, BB, CC, DD). Following habituation, they were shown two new faces in the following pairings

(XX, YY, XY, YX). Whereas 57% of the total fixation of term infants at test was directed to the non-identity pairings, only 46% of that of the preterm infants was so directed. Both groups, however, exhibited significant increments in looking at the new identity pairings (XX andYY) relative to their terminal habituation levels, indicating that the term group, while it had encoded the high abstract attribute of "sameness", had not relinquished attention to the component faces. The preterm infants, on the other hand, had processed only the component information.

Overall, the findings indicate that preterm infants are much less sensitive to the configuration of stimulus elements than are full term infants.

Publications:

Caron, A. J., Caron, R. F., and Carlson, V. R. Do infants see objects or retinal images? Shape constancy revisited. Infant Behav. and Dev. 1: 229-243, 1978.

Caron, A. J., and Caron, R. F. Effects of ecologically relevant manipulations on infant discrimination learning. Infant Behav. and Dev. 1: 291-307, 1978.

Caron, R. F., Caron, A. J., Carlson, V. R., and Cobb, L. S. Perception of shape-at-a-slant in the young infant. Bulletin of the Psychonomic Society, 13: 105-107, 1979.

Caron, A. J., Caron, R. F., and Carlson, V. R. Infant perception of the invariant shape of objects varying in slant. Child Dev. 50: 00-00, 1979.

Caron, A. J., and Caron, R. F. Cognition of relational information and infant risk status. In Friedman, S. L. and Sigman, M. (Eds.): Pre-term and post-term birth: relevance to optimal psychological development. New York: Academic Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00261-03 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Accuracy of Hierarchical Clustering Solutions in Multinormal Mixture Analyses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Craig Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.18	.06	.12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major statistical problem in mental health research has been how to translate subject's scores on a set of dimensions into a meaningful and reliable classification of individuals. This problem is particularly significant since decisions (e.g., assignment to different treatments) should be made on the basis of a subject's entire pattern of scores, rather than scores on a single dimension. The purpose of this research was to develop some new clustering algorithms that include corrections for unreliability, and to compare these algorithms using computer generated data sets. Parametric comparisons between hierarchical clustering algorithms were made, using several evaluative criteria including accuracy, precision, stress, and the cophenetic correlation coefficient. Algorithms using the product-moment correlation as the measure of similarity were superior to those using Euclidean distance, and those algorithms employing empirical Bayesian corrections for unreliability performed better than conventional algorithms.

PHS-6040
(Rev. 10-76)

Project Description:

A major statistical problem in mental health research has been how to translate subjects' scores on a set of single dimensions into a useful classification of individuals. This problem is particularly significant since decisions regarding diagnosis, treatment assignment, and delivery of services must be made about whole individuals, rather than their scores on rating scales, symptom checklists, or questionnaires. Cluster analysis is a family of statistical procedures that is becoming more commonly used in mental health research to produce classifications of individuals from a multivariate data set. Unfortunately, many clustering algorithms are available, different algorithms produce different results, and some algorithms are clearly unsuited for the type of data used in behavioral research. Hence, it is difficult to choose an algorithm for a clustering application, and to place confidence in the resulting typology.

This research compared several of the most widely used hierarchical clustering algorithms on their ability to retrieve the underlying data sets from multinormal mixtures. Five amalgamation rules were analyzed including (1) single or minimum linkage, (2) complete or maximum linkage, (3) average linkage (unweighted pairwise group mean average linkage), (4) centroid linkage (weighted pairwise linkage), and (5) Ward's error sum of squarestechnique. In addition a new algorithm, based on an empirical Bayesian modification of centroid linkage, was developed and evaluated. With this algorithm, after each amalgamation, the resulting centroid is corrected for unreliability and expected regression to the centroid mean, thus producing a more reliable and precisely estimated clustering criterion. The purpose of this research was to supply empirical criteria for choosing a clustering algorithm and measure of similarity, and for deciding whether to standardize data before clustering.

The data sets were 10 multinormal mixtures, generated using Monte Carlo techniques. The number of underlying populations in each mixture ranged from 2 to 5, and the number of variables ranged from 7 to 22. Means for the variables were sampled from the range of 40 to 60, with a measurement error of ± .60 of the standard deviation of the variable. In addition, the ten data sets were prepared in either standardized or unstandardized form, resulting in a total of 20 data sets.

Accuracy of the clustering solution was defined as the agreement between the obtained clusters and the underlying populations in the mixtures. Accuracy was measured using the statistic kappa, a standardized measure of agreement between cross-classifications, and Rand's statistic, a measure based on pair-placements. Precision was defined as the degree to which the obtained clusters approximated the true population parameters of the mixture. Precision was measured by the error sum of squares function. In addition the cophenetic correlation and stress were calculated for each clustering solution.

Based on evaluations using 10 multinormal mixtures, the algorithms could be divided into two groups regarding their performance on these evaluative criteria. The group having high accuracy and precision and low stress included average and centroid linkage algorithms using the product-moment correlation as the measure of similarity, and Ward's minimum variance technique. In addition, the algorithm using the empirical Bayesian clustering strategy was better than conventional clustering algorithms. Complete and single linkage were consistently less accurate and precise than the other algorithms, regardless of the similarity measure used.

This is a final report.

Publications:

Edelbrock, C. Mixture model tests of hierarchical clustering algorithms: The problem of classifying everybody, Multivariate Behavioral Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00263-03 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral Problems and Competencies of Clinic-referred and Nonreferred Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md. 20205

TOTAL MANYEARS: .64	PROFESSIONAL: .32	OTHER: .32

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to compare the behavioral problems and competencies reported for clinic-referred and nonreferred (normal) children, a survey of 1300 randomly selected parents in the Washington, D.C. area was conducted. Home interviewers administered the Child Behavior Checklist to parents of 50 boys and 50 girls at each age 4 through 16. The results have been compared with parental reports of problems and competencies for clinically referred children matched to the nonreferred children for age, sex, socioeconomic status, and race in order to provide a unique epidemiological baseline for research on child psychopathology and the effectiveness of various treatments.

PHS-6040
(Rev. 10-76)

Project Description:

Although there have been a few normative studies of behavior problems in nonclinical groups of children and other studies of problems in clinical groups, there have been no systematic comparisons of problems and competencies reported for broad samples of clinically-referred and nonreferred children using the same instrument during the same period of time. Comparisons of this sort are badly needed in order to determine what kind of problems and competencies typically differentiate "normal" children from those who are recognized as being disturbed enough to warrant professional attention. Because types of problems and competencies are likely to differ with the age, sex, race, and socioeconomic status of the child, the study is designed to compare referred and nonreferred children matched for these variables at each chronological age from 4 through 16.

The data on the referred children have been collected with the Child Behavior Checklist administered in some 25 child mental health settings in the course of developing the Child Behavior Profile described in the Annual Report on Project No. Z01 MH 00244-03 LDP. The data on the nonreferred children were collected by selecting addresses at random within census tracts stratified so as to obtain a distribution similar to the distribution of clinic cases with respect to socioeconomic status and race. Letters describing the survey were mailed to the selected addresses, and recipients were asked to return a postcard if they had no children aged 4 through 16 living with them. An interviewer then went to each address from which no postcard was returned. If a parent consented to be interviewed and if more than one child between the ages of 4 and 16 resided there, a random number table was used to select the child to be described on the Child Behavior Checklist, excluding any who had received mental health services within the previous year.

The scores obtained by the nonreferred children on most behavior problem and social competence items of the Child Behavior Checklist have found to differ at highly significant levels from those of clinically-referred children within each age and socioeconomic group. Although lower socioeconomic parents reported more problems and lower competence on some items than did upper socioeconomic parents, these differences were far smaller than the referred versus nonreferred differences across socioeconomic strata. Racial differences were minimal within referred and nonreferred samples. Separate comparisons for each sex show highly significant differences between referred and nonreferred children on most items, but the problems that best discriminate the groups vary with the age and sex of the children. These data provide a unique epidemiological baseline for further research on child psychopathology and on the effectiveness of various treatment approaches in bringing a child's behavior into the normal range for his or her age.

Publications:

Achenbach, T.M. Clinical research. Yearbook of the National Society for the study of Education, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00265-03 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chronic Malnutrition and Child Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Robert Klein		Institute of Nutrition of Central America and Panama	

COOPERATING UNITS (if any)

Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.75	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is an investigation of the effects of chronic moderate undernutrition on the social, emotional, and cognitive development of children. Six- to eight-year-old children identified as having a history of malnutrition (protein-calorie deficiency) are compared with children who have had more nearly adequate diets. These children are from villages which participated in a longitudinal study from which there are extensive data on food intake, health, growth, and earlier cognitive development. Groups are of very similar social backgrounds. Anthropometry and health and nutritional history are used to assess nutritional status. Standard tests and observations in controlled and natural settings are used in investigating cognitive, sensorimotor, and attentional capacities, and social and emotional behavior. In addition, data are obtained on the children's performance in school, and on the family and village circumstances in which the children live.

Project Description

The research objective is to investigate the effects of a history of chronic moderate undernutrition on the sensorimotor, cognitive, social, and emotional development of children. In general, the behavioral consequences of early moderate chronic undernutrition on the functioning of children have not been adequately investigated. Most studies of malnourished children have been focused on intellective capacities in the early years. Little research has been directed to the effects of nutritional deficit on the child's social and emotional functioning. From the behavioral impairments identified in animal research and reported anecdotally in work with humans, there is the suggestion of functional problems. Failure to sustain attention, lack of impulse control, aimless activity, and heightened emotionality, all of which are reported in animal research, if characteristic of children, could be expressed in learning disabilities in school, impaired coping with environmental demands, disordered emotional responding, and incompetent day-to-day functioning. Specifically, it is hypothesized that chronically malnourished children, compared to their more adequately nourished peers, will show more attentional deficits, apathy, aimless activity, and emotionality, and less curiosity and persistence.

The children, 6 to 8 year olds, have been participants in INCAP's longitudinal intervention research in Guatemalan villages. From this research, there are extensive data on the children's nutritional history, health, growth, and earlier cognitive development. Selection of children is based on (a) their nutritional history (i.e., the best assessment of year to year food intake: the kind and amount of supplement they consumed in the INCAP program and the home food intake), and (b) current anthropometric measures (height-for-age within the Guatemalan population and measures of weight and skinfold). Children who on the combined criteria appear to have had histories of inadequate nutritional intake will be compared with children who have had more nearly adequate histories of nutritional intake. The villages in which the study is carried out will be provided food supplement of enriched flour.

Psychological assessments are made (a) on standard individual tests and (b) in observations of children in small play groups. The group activities are programmed to permit assessment of a range of responses. Assessments will be made on (1) sensorimotor and cognitive variables: attention and information processing, impulse control, reasoning and problem solving; and (2) social-emotional variables: motivation, work efficiency, emotional qualities, attention and processing of social cues. Field observations will be made of the child's home and village environment, and performance and reputation in school are assessed.

Procedures based on a parallel study in the U.S. (Z01 MH 02131-01 LDP) have been adapted to the Guatemalan culture and translations have been made into Spanish. The collection of data and assembling of relevant records on the child are underway and will be completed in the summer of 1979.

Addendum

This project is being carried out by Laboratory staff in collaboration with INCAP. A contract of $25,000 is used to train personnel from the local areas and to support them for the collection of the data. A member of the Laboratory staff is also involved in the field work.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00266-03 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Newborn Interaction with Mothers and Nonmothers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz	Guest Worker	LDP NIMH
Other:	Robert L. Maniello	Guest Worker	LDP NIMH
	Albert R. Hollenbeck	Guest Worker	LDP NIMH
	S. Lawson Sebris	Psychologist	LDP NIMH

COOPERATING UNITS (if any)
Alexandria Hospital, Alexandria, Virginia
Holy Cross Hospital, Silver Spring, Maryland

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.72	.21	.51

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation included three related studies: (1) The effects of 2 types of contact (skin-to-skin or cradling) and 2 durations of contact (15 or 60 min.) between a mother and her newborn infant in the first post-partum hour on the mother's attachment to her infant, mother and infant behaviors during observed interactions, and the infant's behavioral development; (2) the effects of 2 durations of contact (5 or 60 min.) between a father and his infant born by cesarean section in the first 1 1/2 hours post-partum on the father's attachment to his infant, interaction of the father and infant, mother's interaction with the infant, and the infant's behavioral development; and (3) the effects of a program of intense interaction between a nursing student and an infant on the student's attachment to the infant and the infant's behavioral development. Preliminary analyses of study 1 have found differences in both mother and infant behaviors as a result of the duration and type of contact in the first hour after birth.

PHS-6040
(Rev. 10-76)

Project Description:

Recent work has drawn attention to the fact that skin-to-skin post-partum contact between neonate and mother in the first hour (deChateau, 1976; Hales et al, 1977) and extended contact of mother and infant during the first three days (Klaus, Jerauld, Kreger, McAlpine, Steffa, & Kennell, 1972) could result in maternal behaviors denoting attachment to the infant, both in the newborn and in subsequent periods. This research has the primary purpose of replicating and extending studies of the possible effects of immediate post-partum adult-infant contact on the development of the adult's attachment to the infant and the behavioral development of the infant.

This research involved 3 related studies of post-partum contact conditions between an infant and either its mother, father, or a nursing student. Behaviors which may denote attachment were observed during subsequent adult-infant interactions. Infant subjects for all studies were normal, healthy, fullterm newborns from white, intact middle-class families.

In Study 1, mother-infant contact in the recovery room during the first post-partum hour occurred under one of four randomly assigned conditions: 1) 60-minute skin-to-skin contact, where newborn was placed on the mother's abdomen, supported by her, and covered with a blanket 2) 15-minute skin-to-skin contact; 3) 60-minute cradling, where newborn was wrapped in a blanket and cradled in the crook of mother's arm; and 4) 15-minute cradling. Mother and infant behaviors during this initial contact in the recovery area were observed. Observations of mother and infant during a feeding and between feedings were made in the mother's hospital room at 2 days after birth, and again at 28 days during a home visit. The infant's physical and social development was assessed at 2 days by the Brazelton Neonatal Assessment Scale. The mother completed a questionnaire based on the Brazelton Assessment at 2 and 28 days, a diary of mother-infant interaction was collected, and observers rated mother and infant behavior characteristics at 28 days.

In Study 2, the subjects were infants delivered without complications by routine cesarean section, their fathers, and their mothers. Fathers were randomly assigned to either a 5- or a 60-minute contact in a special-care nursery within 1 1/2 hours of the delivery of the infant. Data collection in this study was comparable to that in Study 1, involving father and infant and mother and infant.

The third study involved post-partum contact between infants and second-year nursing students. Each student was involved in a program of intensive interaction for 2-3 days with an assigned newborn. This interaction was governed by a script that called for repeated tactile and auditory stimulations, and repeated assessment of consolability, cuddliness, and visual fixation of the infant. The student also kept a running diary of the infant's states, responses to stimuli, and the student's behavioral assessments. The nursing student was observed twice daily for 15 minutes with this assigned infant and also with a control infant who received no additional contact from the student. The intensive-contact infants and the low-contact control infants were also observed with their mothers for a 15-minute period at 3 days after delivery. In addition, a Brazelton Assessment was made on the infant at 2 days. One focus of Study 3 was whether intensive interactions of nursing-student care-givers with newborns could aid in the development of newborn social behavior. Another aim was to determine if concentrated attention to a particular infant can affect the attachment behaviors of a nursing student to that infant as compared to a control infant with whom there was little interaction.

Data analysis is currently in progress. Preliminary analyses have been completed on the effects of duration and type of post-partum contact between mother and infant (Study 1). Effects have been found in both mother and infant behaviors during observed mother-infant interactions. These effects have been found for both the duration of post-partum recovery room contact (15 or 60 minutes) and the type of presentation (cradling or skin-to-skin) during that contact.

As an example of a result pattern detected to date, mothers who had the initial skin-to-skin contact with the infant were rated at 28 days as showing closer physical involvement with their infants, more satisfaction with their infants' characteristics, and more appropriate behavior in connection with their infants' feeding.

Publication

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00269-02 LDP

PERIOD COVERED October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Assessment of the Outcome of Child Psychiatric Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	David Caine	Clinical Psychologist	Clifford Beers Clinic, New Haven, Conn.
	Lurline deVos	Research Psychologist	" "

COOPERATING UNITS (if any)

Clifford Beers Guidance Clinic, New Haven, Connecticut

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.43	.16	.27

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to assess relations between the patterns of problems and competencies displayed by children referred for guidance clinic treatment and the outcome of two widely offered treatment modalities, namely, individual psychotherapy and conjoint family therapy. The subjects are applicants to a guidance clinic who manifest behavior patterns corresponding to several preselected types and whose therapists judge individual psychotherapy and conjoint family therapy to be equally good treatment modalities for them. If the child and family consent, the therapist administers either psychotherapy or family therapy, as determined by the research design. The course and length of therapy depend upon the child's progress and the therapist's judgment, but an independent evaluator obtains ratings from the parents and the child's teacher at the beginning of treatment and again at 6 and 12 months after intake.

PHS-6040
(Rev. 10-76)

Project Description:

Despite the large number of children in need of mental health services, there have been very few controlled studies of the effectiveness of such services. Even the best of the few existing studies suffer from a fundamental defect: They have assessed the effectiveness of treatment indiscriminately across heterogeneous groups of children. Despite the fact that no single approach to treatment is likely to be uniformly optimal with all kinds of children from all kinds of backgrounds manifesting all kinds of problems, these studies have not been designed to determine what kind of treatment is most beneficial for which children.

The purpose of the present study is to perform a controlled evaluation of the effectiveness of different outpatient treatment modalities with children who are grouped according to demographic characteristics (age, race, sex, socioeconomic status) and according to patterns of behavioral problems and competencies scored on the Child Behavior Profile. The Profile has been developed by Dr. Achenbach as a standardized instrument for describing children's problems and competencies, for identifying overall behavior patterns, and for measuring behavioral change. Cluster analysis of Profile patterns has made it possible to identify a limited number of "types" that characterize the vast majority of children seen in outpatient psychiatric facilities. This typology of Profile patterns provides a basis for classifying children in order to determine which ones benefit most from which type of treatment. In our study, applicants to Clifford Beers Guidance Clinic who are matched with respect to age, sex, socioeconomic status, race, and Child Behavior Profile type receive one of two treatment conditions: individual psychotherapy or conjoint family therapy. The focus is not on which therapy is the better of the two but on differences in outcomes for different types of children who have received the two different types of treatment. Thus, several highly informative findings are possible. For example, it might be found that certain types of children do well under one treatment and poorly under the other. This would provide a basis for more precise prescription of treatment based on child characteristics. Another possible finding is that certain types of children do well under both treatments and other types do poorly under both treatments. This would indicate the need for trying a wider range of treatment modalities with the latter group or for developing new approaches if no existing approach is found to be effective with these children. Whatever the outcome for each group, the fact that children who are similar with respect to Profile type, age, sex, race, and socioeconomic status will have received different treatments according to a systematic research design will permit a far stronger basis for inferring the effects of each treatment with each type of child than is possible without controlled assignment to treatment conditions.

The initial phase of the study includes only boys aged 6 through 11 having any one of four behavior Profile types we have identified for this group. The reason for beginning with this group is that they are by far the most numerous clients of child guidance clinics. Because the number of participating therapists is limited, and because each therapist has continuing responsibility for the children who become part of the study, the chances of obtaining at least 10 subjects in each of the 4 (Profile types) x 2 (treatment types) =8 cells of the research design are best with this group. However, if the research design is completed with this group well within the three years planned for the study, then similar procedures will be followed with girls and/or with boys aged 12 through 16.

Boys aged 6 through 11 who have one of the four target Behavior Profile types and whose therapist judges individual psychotherapy and conjoint family therapy to be equally appropriate for them are assigned to the two treatment conditions in such a way as to produce distributions that are as similar as possible with respect to age, race, and socioeconomic status. This is done by having an independent evaluator score the child's intake Profile after the therapist's clinical evaluation but before the therapist meets again with the family to discuss treatment. If the Profile corresponds to one of the four target types, the independent evaluator determines which of the two treatments should be provided as follows: For the first 10 cases having each Profile type, the evaluator alternates treatment assignments so that the first case having either of two Profile types receives individual therapy, whereas the first case having either of the other two Profile types receives conjoint therapy. Thereafter, the second case of each type receives the treatment that the first case did not receive, and the alternation of assignments to treatment continues until five cases having a particular Profile type have been assigned to each treatment. Because she maintains a record of the age, race, and socio-economic status of each case, the independent evaluator will be able to identify and correct any important differences in the distribution of these variables between cells of the research design.

If a family consents to participate in the study, the data from the Child Behavior Checklist they filled out as a routine part of Beers' clinical evaluation, plus a Teacher's Report obtained with the parents' permission and the therapist's ratings on family functioning, provide the baseline against which behavioral change is measured. Since all the participating therapists are skilled in both types of therapy to be offered, the same therapist will treat the child regardless of which therapy is selected according to the treatment design. The length of treatment is determined by the therapist's clinical judgment and the family's motivation rather than by the research design. Whenever termination occurs, the therapist rates the child and family on various aspects of functioning.

The major focus of the statistical evaluation will be on changes in the behavioral problems and competencies reported by parents, therapists, and teachers from intake until termination and until the 6- and 12-month follow-ups for children of each Profile type receiving each type of treatment. One dimension of analysis will be a comparison of outcomes for children having a particular Profile type but receiving the two different treatment modalities. This will be done in order to determine whether one type of treatment has been more successful than the other type of treatment with children having a particular Profile type. A second dimension of analysis will be a comparison of children having different Profile types in order to determine whether Profile pattern is consistently related to outcome, regardless of type of treatment. The relations between the therapists' intake ratings and the outcome measures will also be assessed in order to determine whether they predict outcome independently of or in conjunction with Profile type and treatment modality. Although a number of children have entered the study, no results will be available until most have completed treatment and the follow-up periods.

Publications:

None

Appendix

Contract # 263MD901924 for $9,844.00 has been used to reimburse Clifford W. Beers Guidance Clinic for the costs of therapist and clerical time, xeroxing, telephone, postage, stationery, office rental, and overhead incurred in the collection of data described in this project summary. The NIMH investigators initiated and designed the study and are performing the statistical analysis and interpretation of data collected by the Clifford Beers staff on their patient population. The data include ratings of their cases by therapists at intake and termination with the clinic, ratings by parents of patients at intake and at two follow-up intervals, and ratings by patients' teachers at intake and follow-ups, obtained with the permission of the patients' parents. Man years = .16, plus reimbursements for the clinic's expenses as indicated.

Contract # 263MD902731 for $9,902.00 has been used to pay for the professional services of Ms. Lurleen deVos, a research psychologist who scores the Behavior Checklists filled out by parents applying to Beers Clinic, determines the Behavior Profile pattern of each case, determines the appropriateness of cases for inclusion in the study, coordinates assignments to treatments, serves as an independent evaluator who obtains therapist, parent, and teacher ratings at intake and follow-ups, codes and collates these and related data on the course and outcome of treatment, and forwards all data in anonymous, coded form to NIMH for analysis. Man years = .75, plus travel expenses for follow-up home visits.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00270-02 LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)
Identification and Validation of Syndromes of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
Other:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other Mental Health Agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS: .73	PROFESSIONAL: .47	OTHER: .26

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to identify and validate syndromes of child psychopathology, we have conducted a literature review of all efforts to derive such syndromes empirically, have made cross comparisons among studies to identify replicated syndromes, and are performing cluster analyses of Child Behavior Profiles in multiple clinical samples. Clusters found to replicate across samples are used to classify children in order to identify demographic, diagnostic, adaptive, and prognostic correlates of the syndromes thus represented.

PHS-6040
(Rev. 10-76)

Project Description:

The lack of an accepted taxonomy for child psychopathology has stimulated many efforts to identify syndromes of behavior problems through multivariate statistical techniques. However, the diversity of subject samples, raters, data collection instruments, techniques of analysis, and methodological flaws made it necessary to do a systematic review and cross-comparison of findings in order to determine whether there is consistent evidence for any syndrome, despite the methodological differences among studies. We found that a distinction between broad-band and narrow-band syndromes seemed warranted because many syndromes derived from first-order factor analyses could be subsumed by a few second-order factors. Broad-band "undercontrolled" and "overcontrolled" syndromes and narrow-band aggressive, delinquent, hyperactive, schizoid, anxious, depressed, somatic, and withdrawn syndromes were found in diverse samples of disturbed children. Two other broad-band and six other narrow-band syndromes were found in a few studies. Test-retest reliabilities and stabilities of syndrome scores were more adequate than interrater reliabilities, which were inversely related to the degree of disparity between types of raters and situations in which they saw subjects. Cross-instrument and cross-population consistencies corroborated some empirically derived syndromes, but the lack of independent criteria for categorizing disturbed children makes it difficult to establish criterion-referenced validity.

Because most efforts to derive syndromes empirically have not been extended to categorization of individual children and the few previous efforts have been limited primarily to the broad-band undercontrolled versus overcontrolled dichotomy, we are applying cluster analysis to Child Behavior Profiles in order to obtain a more differentiated taxonomy. Profile patterns that are replicated through cluster analyses of multiple clinical samples are used to classify children. Differences among the children in demographic characteristics, social competencies, psychiatric diagnoses and the outcomes of clinical contacts are then assessed in order to determine the correlates of the cluster-based taxonomy.

Publications:

Achenbach, T. M.: Psychopathology of childhood: Research problems and issues. J. Consult. Clin. Psychol., 1978, 46, 759-776.

Achenbach, T. M., and Edelbrock, C. S.: The classification of child psychopathology: A review and analysis of empirical efforts. Psychol. Bull., 1978, 85, 1275-1301.

Achenbach, T. M.: Assessment of childhood behavior disorders. In Woody, R.: Encyclopedia of Clinical Assessment. San Francisco, Jossey-Bass, in press.

Achenbach, T. M.: Clinical research. Yearbook of the National Society for the Study of Education, in press.

Achenbach, T. M. Empirical approaches to the classification. In Lachenmeyer, J. R. & Gibbs, M. S. (Eds.): Psychology of the Abnormal Child. New York, Gardner, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02131-02 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Conditions Influencing Interaction between Parents, Nonparents, and Neonates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz, Ph.D.	Guest Worker	LDP	NIMH
Other:	Albert R. Hollenbeck, Ph.D.	Guest Worker	LDP	NIMH

COOPERATING UNITS (if any)
Alexandria Hospital, Alexandria, Virginia
Columbia Hospital for Women, Washington, D.C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIH NIMH ADAMHA Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.77	.36	.41

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This study focuses on the influence of infant characteristics on parent behavior. Specifically, effects on mother's behavior of the attribution to the infant of particular appearance or behavioral characteristics are examined. Three conditions are studied: (1) Mothers are asked to provide infants with "love" or "comfort" --both abstract examples of nurturance. Analyses are made of the mother-infant interaction to which each abstraction reduces. (How these patterns of interaction relate to situational and demographic variables is also examined.) (2) Similar observations are made of hospital nurses when they interact with infants arbitrarily labeled male or female. (3) Following a nominal examination of the infant, the investigator makes a statement to the mother emphasizing a transient infant characteristic. Parental and infant responses (e.g., hugs, smiles, verbalizations, kisses, squeezes) are assessed.

PHS-6040
(Rev. 10-76)

This study focuses on the influence of infant characteristics on parent behavior. Specifically, effects on mother's behavior of the attribution to the infant of particular appearance or behavior characteristics are examined in three related studies. In the first study mothers' response patterns when they are instructed on how to interact with infants were investigated. Mothers are asked to provide infants with "love" or "comfort"--both abstract examples of nurturance. (Within-subject control observations are made by having each mother "get to know your infant".) Nurturance has been an important variable in descriptions of infant development; yet, terms such as "love" and "comfort" are ambiguous as well as idiosyncratically used. We are examining nurturance in a more direct fashion. From our observations, we hope to develop a taxonomy of adult behaviors to infants that define abstract terms like "love" and "comfort".

An hypothesis about adult behavior that persists in the child-rearing literature is that adults respond differentially to children depending on the child's sex, and that this differential treatment, in turn, explains later differences between the behaviors of boys and girls. The aim of our second study is to compare the response patterns of adults to infants, when those infants are arbitrarily labeled male or female. For half of the cases, hospital nurses are told the true sex for a stranger infant with whom they are to interact; for the other half of the cases, the nurses are told the incorrect sex.

Parent behavior to infants also may be influenced by messages communicated to parents by persons having contact with their baby. The aim of the third study is to investigate how adult-infant interaction may be influenced by an attribution to the infant of particular transient behavioral characteristics. Thus, mother-child interaction is first observed under standard conditions. Then the staff member remarks to the mother that the infant is (a) "more active" or (b) "less active" than he or she was during a time when the infant had been alone with the staff member. Observations of resulting mother-infant interaction are made and comparisons are made between patterns of interaction observed before and following the observer's comment.

Work on data collection has been completed for study one (48 subjects) and three (52 subjects) and is nearing completion for study two (14 out of 24 subjects). Data reduction and analyses are underway.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02132-02 LDP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Children's Misdeeds and Parental Disciplinary Practices

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .78	PROFESSIONAL: .25	OTHER: .53

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this research is to examine the roles of children in influencing the kinds of discipline used by parents. The sample is 24 middle class mothers and their children who are in the second and third years of life. The data are reports by mothers trained in observation techniques, and ratings made in the home by investigators. Children's misdeeds and mothers' reactions to these misdeeds were investigated. Categories of misdeeds were harm to people, harm to property or objects, and "lapses" in self control (e.g., temper tantrums). Forms of discipline included reasoning, verbal prohibitions, physical punishment, love withdrawal, guidance in appropriate behavior. Analyses are focused on (a) the possible ways in which the specific types of misdemeanors influence parents' choices of disciplinary practices and (b) association between general temperamental characteristics of children and parental discipline.

PHS-6040
(Rev. 10-76)

Theories of socialization most often have been formulated in terms of a unidirectional model: The influences on children's behavior of different parental rearing and disciplinary practices (e.g., physical punishments, reasoning, withholding of resources) are investigated. More recently, increased attention has been given to the role of child characteristics in determining parents' reactions to their children. The aim of this research is to examine the roles of children in influencing the content of parental disciplinary practices. Two questions are addressed: Do specific types of misdemeanors by children tend to provoke characteristic forms of discipline from parents? Are general temperamental and affective characteristics of children associated with particular forms of discipline from parents?

The sample is 24 middle class mothers and their children ages 1 to 2½ years. Data were collected over a nine month period. The data on temperament are obtained in ratings made by investigators during regularly scheduled home visits. The data on misdemeanors and discipline are mothers' dictated observations of specific incidents of misdeeds by their children and disciplinary reactions to these misdeeds. (For descriptions of the training and reporting procedures for mothers see Project Z01 MH 00242.) Incidents were coded for type of misdeed and type of discipline. Types of misdeeds included harm to people, harm to property or object, and lapses in self control. Forms of discipline included reasoning, verbal prohibitions, physical punishment, love withdrawal, and guidance in appropriate behavior.

The data have been collected and coded. Data analysis and preparation of a manuscript are under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02133-02 LDP

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Stability and Change in Children's Responsiveness to Others' Emotions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.55	PROFESSIONAL: .55	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Responsiveness of young children to the feelings and emotions of others is examined in relation to the following questions: (1) How consistent over time are individual patterns of reaction to others' emotional distress? Assessments of children in the second year of life are compared with assessments four years later. (2) As a group, how do children's responses to others' emotional distress compare at the two age periods? (3) Are associations between maternal rearing characteristics and children's empathy and altruism similar at the two ages? Data on children's altruism and parental child rearing practices are obtained through naturalistic observations from parents and investigators, and in laboratory assessments. While some forms of altruistic behavior change with age, the intensity of empathic expression at the two ages is not systematically different. There is consistency from age 1½-2½ to age 6-7 in the majority of individual children's mode of responding (e.g., compassionate, indifferent, avoidant). There is consistency over time, also, in some maternal rearing practices, particularly in practices that reflect high expectations and standards regarding interpersonally responsible behavior in children.

PHS-6040
(Rev. 10-76)

Project Description:

The development and socialization of emotional responsiveness of young children to the feelings and emotions of others are investigated in order to address the following questions: (1) How consistent over time are individual reactions to others' emotional distresses? Children studied in the second year of life (Z01 MH 00242-05) are again studied at seven years of age. (2) As a group, how do children's responses to others' emotional distresses compare at the two time periods? (3) Are stabilities and changes in children's responsiveness to others' emotional distress related to parental rearing practices? Parents are trained in observing and reporting (dictating) narrative accounts of children's responses in naturally occurring situations of distress. Standard laboratory assessments are also made.

Findings:

Children at age 7, compared with age 2, show more cognitive skills in coping with others' emotions. They are able to deal with more abstract kinds of distresses. They pick up more subtle cues and take into consideration feelings of the other person that are beyond the immediate observable events. Aside from these changes, there is a near absence of a developmental progression in the judged intensity of affective expressive response to distress. Also, there is no difference in frequencies of helping behavior in the face of distress by the two- and seven-year-olds. In this sense, there are no startling stages in empathy in early childhood.

Stable individual patterns in empathic characteristics are identifiable among the 1½ to 2½ year olds. There were 2-year-olds whose profiles of response emphasized avoidant and intolerant reaction; those who were distinguished by analytical and intellectualized responding; and others who were intensely affective and compassionate. Still others were nonresponsive and nondescript in reactions to distress. These individual patterns carry over to ages 6-7 in a significant number of children, demonstrating continuity or predictability across developmental periods in children's coping with others' emotions.

Developmental profiles are being further analyzed. Stability and change in children's responding to distress also will be examined in relation to parental rearing practices, particularly those practices that reflect strong principles and high standards regarding what is expected in interpersonally responsible behavior in children.

Publications:

Waxler, C. Z., Yarrow, M. R., & King, R. A.: Child rearing and children's prosocial initiations toward victims of distress. Child Dev., 1979, 50.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02134-02 LDP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Developmental Changes in the Understanding of Pictures Representing the Production of Sound

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Research Psychologist	LDP NIMH
OTHER:	Marguerite B. Stevenson	Research Psychologist	Univ. of Wisconsin Madison, Wis.

COOPERATING UNITS (if any)

University of Wisconsin, Madison, Wisconsin 53706

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.17	.68	.49

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this investigation are examined age changes in the ability to interpret correctly pictorial representations of sound production. Sound cannot be captured in a three dimensional picture; however, the production of sound can be represented by various techniques: (1) Postures. Some postures suggest that sound is being produced; for example, a person may have his mouth open and lean toward the listener. (2) Context. Musical instruments, tools, and other objects are understood to make sound when they are held or moved in certain ways. (3) Arbitrary methods are used to suggest sound production. These methods include balloons containing words, lines, or musical notes drawn next to the source of sound. Pictures using the three techniques for representing sound and matched pictures without indications of sound will be shown to subjects between 2 and 20 years of age. It is expected that there will be an interaction between age and the pictorial technique used when the correctness of interpretations is examined.

Project Description

A correct reading of representational pictures involves the ability to detect an equivalence between pictorial forms and their real referents. Yet, there are many differences between pictures and the reality they represent. Thus, a picture is a flat surface while our world is three dimensional. The pictorial shapes very rarely match the size, color, and texture of the objects represented. Movement must be represented statically and the production of sound must be represented without sound. Despite these differences between pictures and reality, pictures are widely used as a means of communication.

In this culture, children are introduced to picture books when they are as young as six months. Yet, the materials presented in picture books are not always geared to the child's picture reading abilities. For example, Friedman and Stevenson (1978) found that contemporary children's picture books make use of pictorial movement information which young children (kindergarten, first grade) ignore when classifying pictures as showing or not showing movement. In a small survey of 11 picture books with a total of 248 pictures, 39 pictures represented sound production. Currently we do not know to what extent the children for whom these pictures were designed are capable of understanding the pictures.

In this investigation, we will examine age changes in the ability to interpret correctly pictorial representations of sound production. Sound cannot be captured in a three dimensional picture; however, the production of sound can be represented by various techniques. Some pictures suggest sound production by posture. The speaker may have his mouth open as he/she looks and gestures toward the listener. Some pictures of sound production show musical instruments, tools and other objects capable of making sound. In order to understand these pictures, it is necessary to understand the ways in which these objects make sound in the environment. Other pictures of sound production use arbitrary symbols for sound. For example, in medieval art, words are drawn on ribbons coming from the mouths of angels. In contemporary cartoons, balloons with words or musical notes are drawn near mouths. We hypothesize that pictures using the above techniques vary in the degree to which they suggest that sound is being produced. Consequently we predict that the understanding of the postures suggesting sound requires the least pictorial acculturation while the understanding of arbitrary indicators of sound requires the most acculturation. Pictures using the three techniques for representing sound and matched pictures without indication of sound will be shown to subjects between 2 and 20 years of age. It is expected that there will be an interaction between age and the pictorial technique used when the correctness of interpretations is examined.

The study will provide some of the evidence that will help design appropriate pictorial materials for children of different ages. This is an important objective since in this culture pictures are important means of introducing children to the possibility of visual representation and to reading--the decoding of visual patterns which are often quite arbitrary. The procedures and stimuli were developed, and we are currently in the process of data collection.

Publications:

Friedman, S. L. and Stevenson, M. B. Perception of Movement in Pictures. In Hagen, M. A. (Ed.): What, Then, Are Pictures?: The Psychology of Representational Art. San Francisco, Academic Press, 1979. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02135 -02 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Children's Responses to the Emotions of Others: Naturalistic and Experimental Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
OTHER:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.60	.45	1.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The focus of this research is the emotional development of children in families in which one of the parents is suffering from an affective disorder. These studies are an extension of a program of research in which children's encounters with the emotions of others are examined. In the present study emphasis is given to the chronic aspects of emotional encounters. Families to be included are those in which a parent has (Study A) a history of bipolar depression, or (Study B) a parent has been diagnosed schizophrenic. Children from families with no known pathology will also be studied. Two sources of data are used: The first is parental observations, reported in narrative, of emotional incidents and child responses (See Z01 MH 00242 for procedures). The second source of data is standard situations in which mother and child interactions will be observed in the laboratory in activities involving a range of caregiving functions. Cognitive measures of the children will also be obtained in the laboratory. Data collection is underway on Study A. Study B is in a planning stage.

PHS-6040
(Rev. 10-76)

Project Description:

The focus of this research is the emotional development of children in families in which one of the parents is suffering from an affective disorder. These studies are an extension of a program of research in which children's encounters with the emotions of others are examined. Previous studies have been of children in families that are free of severe pathology, and of children in the first two years of life. Emphasis has been on discrete emotional events, with little attention to a systematic conceptualization and sampling of the affective environment of the family. In the present study greater attention is given to the chronic aspects of emotional encounters. Also, children of several age levels will be sampled.

Study A, currently underway, consists of families in which one of the parents has a history of bipolar depression. Observations were begun when the children were 12 months of age and are being continued for six to nine months. Older siblings will also be included. Study B, comparing children of schizophrenic mothers with children from families with no known pathology, is being planned.

Two sources of data are used: The first is parental observations, reported in narrative, of emotional incidents and child responses (See Z01 MH 00242 for procedures). In Study A, both parents will provide these reports; in Study B, mothers only will provide them. The second source of data is standard situations in which mother and child interactions will be observed in the laboratory in activities involving a range of caregiving functions. Cognitive measures of the children will also be obtained in the laboratory.

Data collection is underway on Study A. Study B is in a planning stage.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02136-02 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Prosocial Interaction Among Physically Handicapped and Nonhandicapped Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karabelle A. L. Pizzigati	Guest Worker	LDP	NIMH
OTHER:	Gale Inoff	Psychologist	LDP	NIMH
	Howard A. Moss	Research Psychologist	LDP	NIMH
	Ronna Finer-Berman	Guest Worker	LDP	NIMH

COOPERATING UNITS (if any)
Montgomery County Public Schools

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.79	.67	.12

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent legislation indicates that there will be greater integration (i.e., mainstreaming) of handicapped children. The resulting social adjustment of handicapped and nonhandicapped children to each other is a major concern. The purpose of this project was: (1) to compare, in terms of their social, play, and affective behaviors, handicapped and nonhandicapped children, (2) to investigate the effects of being from a mainstreamed school, and (3) to study the effects of having a handicapped child in a play group of nonhandicapped children. Experimenters observed and coded the behaviors of orthopedically handicapped and nonhandicapped children ages 5-10 years in small group play sessions. Nonhandicapped children were found to be more often involved in the social exchanges and group activities than were the handicapped children. Having been to a mainstreamed school positively affected the way nonhandicapped children interacted with a handicapped child. Other than a statistically nonsignificant tendency for the presence of a handicapped child to inhibit the group, groups with and those without a handicapped child were highly similar in those social, play, and affective variables assessed.

Project Description:

In the past, handicapped and nonhandicapped children have been segregated in many settings. Recent legislation, however, indicates that there will be increasing integration (i.e., mainstreaming) of handicapped children, particularly in public school settings. Social adjustment issues, therefore, are extremely important for both nonhandicapped as well as handicapped children.

The purpose of this project was: (1) to compare, in terms of their social, play, and affective behaviors, handicapped and nonhandicapped children, (2) to investigate the effects of being from a mainstreamed school, and (3) to study the effects of having a handicapped child in a play group of nonhandicapped children.

Children ages 5-10 were observed in groups of 6. Twenty groups were seen. Group composition consisted of either 1 handicapped child and 5 nonhandicapped children, or 6 nonhandicapped children. Of the groups having a handicapped child, some of the groups had nonhandicapped children from a mainstreamed school and some had nonhandicapped children from a non-mainstreamed school. The handicapped children were orthopedically handicapped.

The children were observed on measures such as social participation, friendly interaction, negative interaction, helping behavior, task involvement, and happy affect. Some measures were assessments of group functioning; others were assessments of individual children within the group.

Comparisons of nonhandicapped and handicapped children indicated that the nonhandicapped children were more often involved in social exchanges and group activities than were the handicapped children. However, for many variables (e.g., happy affect, negative interaction), there were no differences between handicapped and nonhandicapped children.

Comparisons were made between groups of mainstreamed and non-mainstreamed children, each having one handicapped child and five nonhandicapped children. For most variables, no differences were found; for variables assessing interaction between the handicapped and nonhandicapped children (e.g., initiating and responding to the handicapped child, helping the handicapped child), mainstreaming effects were evident. Having been to a mainstreamed school seemed to contribute toward attending to and showing prosocial behaviors to the handicapped child.

Comparisons were made between groups having a handicapped child and groups with only nonhandicapped children. Although there was a slight tendency for the presence of a handicapped child to inhibit the group, in general, the groups were very similar and those differences which were found were not statistically significant.

It also appeared that the degree to which a handicapped child was ignored was related to the degree to which he or she was passive and failed to initiate interactions. Those handicapped children (like nonhandicapped children) who made demands on the group were less likely to be ignored.

A manuscript is being prepared for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02137-02 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Constancy and Change in Personality Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Elizabeth J. Susman	Research Psychologist	Pennsylvania State University University Park, Pa.

COOPERATING UNITS (if any)
Pennsylvania State University
University Park, Pa. 16802

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.64	.47	.17

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A long standing and pervasive assumption held by lay persons and psychologists alike is that an individual's behavior is generally predictable across a variety of situations and over time. A number of longitudinal studies have been conducted over the past 50 years to test the developmental hypothesis concerning the stability of personality. The purpose of this project is to review and integrate the findings from the various longitudinal studies concerning the evidence for stability and change in personality over the life span. Attention is given to the assumptions associated with assessing stability and change and to the methodological and psycho-social conditions that influence whether stability or change is observed.

Project Description:

The emergence and course of personality characteristics over time is of considerable interest to developmental psychologists. Does a particular behavior continue to characterize the individual in a consistent manner over long periods of time? Are there predictable developmental transformations? Do certain earlier behaviors presage later, but phenotypically different, personality patterns? Are certain personality characteristics ephemeral and are others immutable; and, if so, what factors influence change? It seems patently evident that these questions can only be answered through the pursuit of long term longitudinal studies. Twelve major longitudinal studies have been reviewed with respect to the above questions.

The personality variables examined in this review include affiliation, aggression, achievement, heterosexual behavior, sex role interests, temperament, behavioral style, dependency, passivity, psychological reactions related to stress, and maladaptive behavior. In general, little stability appears over the early months and years of life. Increased age was associated with higher and more significant stability coefficients for most of the personality variables that were studied. A number of personality variables (e.g., achievement, recognition seeking, dependency, aggression, and sex role interests) are significantly correlated with cognate variables assessed during the early adult years. There is more evidence of stability between these personality measures taken within the adult years. However, the strongest relations tend to occur between personality characteristics of contiguous ages with the magnitude of the correlations diminishing as the time intervals between assessments increase.

Socially sanctioned behavior tended to show the greatest stability, whereas attitudes and values that are often associated with specific reference groups and ephemeral cultural positions show low stability. For example, achievement and recognition seeking behavior exhibited stability from childhood through adulthood, starting with the early school years. Dependency behavior, which tends to be culturally sanctioned for females, was stable only for this sex from middle childhood on. On the other hand, aggression was stable just for males over the same span of time.

Publications:

Moss, H. A., and Susman, E. Constancy and Change in Personality Development. In Kagan, J. & Brim, O. (Eds.): Constancy and Change in Human Development, 1979. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02138-01 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Undernutrition and Behavioral Competence in School-age Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian R. Yarrow	Chief	LDP NIMH
Other:	David E. Barrett	Research Psychologist	LDP NIMH
	Robert Livingston	Professor of Neurosciences	University of California San Diego
	Mary Z. Bell	Staff Research Associate	" "
	Elizabeth Stern	Staff Research Associate	" "

COOPERATING UNITS (if any)
Department of Neurosciences, School of Medicine, University of California at San Diego, LaJolla, California 92037

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .85	PROFESSIONAL: .40	OTHER: .45

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is an investigation of the cognitive and social competences of children who have a history of moderate to severe undernutrition and currently have a marginally adequate diet. All children are from the same poor urban circumstances. The sample is approximately 120 seven-year-olds. The children are predominantly Anglos living in Southern California. Three groups of children are studied: (a) children at or below the tenth percentile in height for age and with evidence of a history of undernutrition, (b) children at or below the tenth percentile but not undernourished, (c) children at or above average height for age and not undernourished. Anthropometric measures of child and family, nutritional and health history and status of mother and child, and blood analyses are the bases for classification. Assessments include measures of sensory-motor, cognitive, and attentional capabilities; of impulse control and affective characteristics; of goal-directed and work effective activities; and of social interactional characteristics. Data collection is underway and will be completed in September, 1979.

Project Description:

This is a study of children who have a history of moderate to severe undernutrition and currently have a marginally adequate diet. Cognitive and social competences, it is hypothesized, are impaired by undernutrition experienced in the early years. Based on animal research and on findings on severe childhood malnutrition, impairments are expected in attentional and cognitive abilities, in motivation and goal-directed behavior, and in emotional control. Learning disorders and difficulties in social relationships can be derived from these impairments.

The children in this study are 120 seven-year-olds, mostly Anglos, living in poor urban areas of Southern California. All families are below a poverty income level.

The child's nutritional history and status are determined on the basis of height for age, weight, skinfold, nutritional and health history and status of mother and child, and blood analyses. Based on the combined criteria, children are assigned to one of three groups: (a) small-for-age (tenth percentile or below) with a history of undernutrition, (b) small-for-age (tenth percentile or below) without a history of undernutrition, and (c) average or above height for age and without a history of undernutrition.

The children are tested on the WISC, Bender-Gestalt, Lindamood, and Bruininks-Oseretsky. There are experimental tests of social and emotional behavior. The children are seen also in small groups in two half-day sessions in which there are measures of the social and emotional variables indicated above.

Each family is visited to obtain further data on the home and neighborhood. In visits at the end of the study, the family is given information about the child's physical health and psychological status, and is provided with referrals to professional help in cases of problems.

To date, about a third of the children have been given the entire set of assessments. The remaining data collection will be completed in the summer of 1979.

Addendum:

This project is carried out off-site. The data collection is performed by staff of the University of California, San Diego, on a contract of $30,000. This includes case finding, screening, nutritional history and status, and the psychological measurements.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02139-01 LDP

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Social Cognition and Social Behavior in Situations of Emotional Distress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Z. Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.58	PROFESSIONAL: .35	OTHER: 1.23

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children's capabilities in coping with the emotional distress of other persons are investigated. Two sets of information, one of cognitive data and one of social behavior, are obtained on normal, 5-7 year old children (N = 22). The Peabody Test of mental ability, role-taking tests (Flavell, Selman), and interpretations of incidents of emotional distress (eight incidents, each portrayed in a 3-picture series) are examined in relation to each other, and in relation to the child's responding to distress incidents (naturally occurring incidents observed and reported by the mother (MH-00242) and to standard simulated incidents). Data collection is completed and analysis is underway.

Project Description:

Children's capabilities in coping with the emotional distress of other persons are investigated. Although role-taking abilities and prosocial behavior have been studied extensively as separate issues, their investigation together has been mainly in laboratory studies in which very limited samples of role-taking and prosocial behavior have been examined in relation to one another. Therefore, there is little basis on which to develop understanding of the interdependencies of these processes. The focus of this research is on these interdependencies, making use of substantial response sampling, covering an extended period of naturalistic data collection and a 1½ hour laboratory session.

Two sets of information, one of cognitive data and one of social behavior, are obtained on normal, 5-7 year old children (N = 22). The Peabody Test of mental ability, role-taking tests (Flavell, Selman), and interpretations of incidents of emotional distress (eight incidents, each portrayed in a 3-picture series) are examined in relation to each other, and in relation to the child's responding to distress incidents [naturally occurring incidents observed and reported by the mother (MH-00242) and to standard simulated incidents.] Data collection is completed and analysis is underway.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02140-01 LDP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Interrelations of Early Cognitive Development and Empathic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH
Other:	Carolyn Z. Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.49	.52	.97

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research objective is to examine the development of empathy and prosocial behavior in relation to progressive differentiation of self and other in the second year of life. Data on children's empathic capabilities are taken from mothers' systematic observations of their children's responses to naturally occurring emotional incidents in the home environment. Understanding of self and other is assessed by a Self Recognition Scale (children's reactions to their own mirror image) and an Agent Use Scale (understanding of other persons as independent agents in the context of pretend play). An Object Permanence Scale will also be administered as a standard measure of cognitive development in the sensorimotor period. Thirty children are studied from 10 months of age until two years. Data collection is under way.

PHS-6040
(Rev. 10-76)

Project Description:

Empathy is thought to be one of the primary motives underlying altruistic, helping, cooperative, and other prosocial behaviors (Bryan, Hoffman, Stotland). It has further been suggested that the development of empathy is related to the progressive differentiation of self and other in the second year of life (Hoffman). Clinical evidence suggests that severe deviations in the individuation process may result in pathological and/or antisocial behavior in later life (Bowen, Bowlby, Mahler).

The present study focuses on the hypothesized relation between the understanding of self and other and the development of empathy and prosocial behavior. Two scales assessing the understanding of self and other are administered to 30 infants every two months beginning at 10 months of age and continuing to 24 months. The scales are given in the infants' homes. Empathic prosocial behaviors are assessed from systematic maternal observations in the manner used by the present investigators in previous studies (Z01 MH 00242-05).

In the Agent Use Scale (Watson & Fischer), the experimenter demonstrates three standard actions in relation to self and other (a doll). Then the children are observed in play to determine whether they imitate the demonstrated action in relation to self or other. Of particular interest is the extent to which children give evidence of understanding others as independent agents. It is predicted that the development of children's understanding of other persons' actions as independent of their own actions (as assessed in this task) will parallel the development of the understanding that other persons' distress is independent of their own distress (as determined by the maternal observations described above).

In the Self Recognition Scale (Bertenthal & Fischer), the infant is seated in front of a full length mirror and given a series of self-recognition tasks. For example, a spot of rouge is put on the infant's nose, and the question is whether or not the infant recognizes that he/she has a spot on her/his nose from seeing the reflection. It is predicted that the development of self-recognition will also parallel the development of empathic and prosocial behavior.

An Object Permanence Scale (Uzgiris & Hunt) is given as a standard measure of sensorimotor development. This scale consists of 15 graded tasks in which infants are required to find a hidden object under increasingly difficult circumstances. It is predicted that scores on this scale, designed to measure children's understanding of the physical world, will not be as closely related to the development of empathy as scores on the Agent Use and Self Recognition scales, which are intended to measure the processes which presumably underly empathic development. Data collection is under way.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02141-01 LDP

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Assessing the Progress of Children in Residential and Day Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach,	Research Psychologist	LDP NIMH
Other:	Dr. Joan Barber	Director	Hillcrest Children's Center Washington, D.C.

COOPERATING UNITS (if any)

Hillcrest Children's Center, Washington, D.C.

LAB/BRANCH Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, MD 20205

TOTAL MANYEARS: 1.33	PROFESSIONAL: .22	OTHER: 1.11

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relations among ratings--made by parents, teachers, child care workers, and an independent observer--of the behavioral problems and competencies of children in residential and day psychiatric treatment; (2) to determine the relations between children's initial behavior patterns as reported by these sources and the children's progress in treatment. The initial ratings are made by parents, Hillcrest staff, and the independent observer at the beginning of the academic year and again in the late spring. Relations between initial behavior patterns--derived from ratings by the various sources--and children's progress--as measured by the follow-up ratings and indices of academic functioning--will be compared with similar data on out-patient samples to determine the prognostic validity and generality of the initial behavior patterns.

Project Description:

Because judgments of children's behavioral problems and competencies are inevitably influenced by the conditions under which the children are observed and by the observers' relationships to the children, it is essential to identify areas of agreement and disagreement among observers and to determine the best sources of observations for assessing psychopathology, making treatment decisions, and evaluating change in disturbed children.

The purpose of this project is to extend to children in day and residential treatment the methods of assessment we have developed and applied to children treated on an outpatient basis (Project No. Z01 MH 00259-02). The subjects are 40 children aged 6 to 13 who are in treatment at Hillcrest Children's Center in Washington, D. C. The rating instruments are versions of the Child Behavior Checklist (Project No. Z01 MH 00244) that have been developed for raters having different relationships to the children (parents, child care workers, teachers, direct observers).

The design of the study entails obtaining behavioral ratings from the following observers: Ratings on the Child Behavior Checklist from the child's parents if the child lives at home or from child care workers if the child lives at the treatment center; ratings on the Teacher Report Form from the child's teacher and teacher's aide; and direct observational ratings from a trained observer who observes the child for six 10-minute periods in class and six 10-minute periods at recess. During each 10-minute time sample, the observer writes a narrative description of the child's behavior. From this narrative, she then rates the child on behavior problem items drawn from the Child Behavior Checklist, using 4-step response categories. She also scores on-task behavior at 1-minute intervals within each 10-minute sample.

The ratings are made in the autumn and again six months later in order (a) to determine agreement among the various observers at the beginning of a school year and again at the end of the school year, and (b) to determine which source or combination of sources of ratings best predicts outcomes over the course of the school year. In addition to behavioral ratings, achievement and cognitive measures will be used as indices of improvement.

It is expected that comparison of changes for children differing in initial behavioral patterns will provide a basis for identifying those who do and those who do not benefit from treatment as currently rendered. Comparisons will also be made with findings for outpatients in order to determine whether the predictive power of initial behavior patterns is similar for inpatients and outpatients. Data collection is scheduled to be completed and the analyses are to begin during the current fiscal year.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02142 -01 LDP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Children with Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Beatrix Hamburg	Medical Officer (Research)	LDP NIMH
	Blanche S. Jacobs	Research Psychologist	LDP NIMH
	Sandra Weiss	Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Community Hospitals
National Institute of Child Health and Human Development
National Institute of Arthritis, Metabolism and Digestive Diseases

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 1.29	PROFESSIONAL: .86	OTHER: .43

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives of this research are: (1) to identify attitudes, concerns, and response patterns that children and their parents exhibit when it is discovered that the child has juvenile diabetes and (2) to trace the course of these attitudes, concerns, and response patterns over the first year of the illness. The research involves the study of the two age groups for which the occurrence of juvenile diabetes is greatest: 4-7 year olds and 12-14 year olds. Control groups of healthy children are also seen. Data will be collected in the form of behavioral observations, interviews, and/or standardized tests at the onset of illness, at 4-6 weeks, at 6 months, and at 12 months after onset. This research is considered a prototype for how children in general cope with stress.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The focus of this research is on coping patterns and the acquisition and use of skills in dealing with a stressful life event. Children with serious chronic illness experience discernible and standard stress. This situation is used to study coping patterns. Specifically, the objectives of this research are: (1) to identify attitudes, concerns, and response patterns that children and their parents exhibit when it is discovered that the child has juvenile diabetes and (2) to trace the course of these attitudes, concerns, and response patterns over the first year of the illness, at which time a relatively stabilized style of reacting to the illness should have emerged. This research is considered a prototype for how children in general cope with stress since it is assumed that dealing with juvenile diabetes is a special and standardized life situation that dramatizes developmental processes that are characteristic of all children.

The research involves the study of the two age groups for which the occurrence of juvenile diabetes is greatest: 4-7 year olds and 12-14 year olds. Data, in the form of behavioral observations, interviews, and/or standardized tests, will be collected in the hospital or home at the onset of illness, at 4-6 weeks, at 6 months, and at 12 months after onset. By one year, modal patterns of responding to diabetes should be evident and information about the conditions that contribute to these patterns should be available.

A control group of adolescents will be included in whom the onset of juvenile diabetes occurred during the 4-7 age period. These subjects will be compared to the 12-14-year-old experimental group at one year after onset of the illness to determine whether certain behavior patterns are a function of juvenile diabetes developing during adolescence or whether these are characteristic patterns of reacting to diabetes during the adolescent years, regardless of the age of onset.

Control groups of healthy children between 4 and 7 years of age and 12 and 14 years of age and their parents will also be studied. Healthy children will be recruited from among friends of the diabetic families to facilitate matching the healthy and diabetic samples on dimensions unrelated to the illness (e.g., social class, values, etc.). Control groups will be seen for one session and will be administered the same procedures (exclusive of items dealing with health or medical aspects of diabetes) as the diabetic samples in order to evaluate whether the observed behaviors are a function of experiencing a serious chronic childhood illness or reflect typical developmental patterns.

This study places an emphasis on coping behavior and focuses on three types of psycho-social stress experienced by children with juvenile diabetes: stress relating to monitoring and regulating their blood sugar levels on a daily basis for the remainder of their lives, stress associated with apprehension over possible later complications, and interpersonal stress associated with having juvenile diabetes. The following illness-related variables also will be emphasized in the research: age of the child at time of onset of the illness, the pre-illness psycho-social functioning of the child and the parents, the severity of the initial bout with the illness and the skill in the management of the situation by the attending medical personnel, and the availability of support systems.

This study is in a preliminary stage of procedural development.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02143-01LDP

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychosocial and Biomedical Interactions in Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Beatrix A. Hamburg	Research, Medical Officer	LDP NIMH
Other:	Gale Inoff	Psychologist	LDP NIMH
	Sandra Weiss	Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Neonatal and Pediatric Medicine Branch, NICHD, NIH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.54	1.21	.33

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Juvenile diabetes is a major cause of morbidity, mortality, and great emotional suffering. Additionally, psychosocial factors and emotional stress are thought to be influential regarding adherence to treatment regimens as well as complications in the course of the illness. The study of juvenile diabetes is appropriate as a model for understanding the interactions of biomedical and psychosocial factors in affecting medical and psychological well-being. Patients in the study are 60 metabolically labile diabetic patients (brittles), a comparison group of 60 non-brittle diabetics, and a comparison group of 60 normal subjects. Patients are between ages 10-22 years. In this project, the states and changes in the psychosocial and biological systems are viewed as interdependent and influencing each other at all times. Data are being collected in both systems. Emphasis also is placed on strategies of coping with life stress and social support as a moderator of stress. Attempts are being made to differentiate sub-sets of patients for whom psychosocial and biomedical variables cluster and, in turn, to trace the health consequences for individuals with differing patterns of response.

PHS-6040
(Rev. 10-76)

Project number Z01 MH 02143-01

"Psychosocial and biomedical interactions in juvenile diabetes"

Juvenile diabetes is a major cause of morbidity, and morality, and of emotional suffering for patients and their families. Additionally, psychosocial factors and emotional stress are thought to be influential regarding adherence to treatment regimens as well as complications in the course of the illness. The study of juvenile diabetes is appropriate as a model for understanding the interactions of biomedical and psychosocial factors in affecting medical and psychological well-being. Because of the clash of the specific demands imposed by the illness and those inherent in the developmental period, studying diabetes in adolescents should be especially valuable.

Patients are 60 metabolically labile diabetic patients (brittles), a comparison group of 60 non-brittle diabetics, and a comparison group of 60 normal subjects. Patients are between ages 10-22 years. Behavioral observations, interviews, and standardized tests are administered while the children are inpatients for one week at the NIH Clinical Center. (Parents, siblings, and doctors of patients also provide information about the patients.)

The states and changes in the psychosocial and biological systems are viewed as interdependent and influencing each other. Data are being collected in both systems. Attempts are being made to differentiate sub-sets of patients for whom psychosocial and biomedical variables cluster in distinctive patterns of behavioral-neuroendocrine responses to stress and, in turn, to trace the health consequences for individuals with differing patterns of response. Emphasis also is placed on classifying strategies of coping with life stress and assessing social support as a moderator of stress. The independent indices of organismic arousal due to stress will be cortisol levels and heart rate.

Some specific areas being studied are:

(1) The effects on mood and behavior of moderate changes in glucose and other stress-related metabolites.

(2) Individual differences in the range and pattern of metabolic changes that result from situations of both emotional arousal and physical activity.

(3) Patterns of match and dysfunctional mismatch between the medical and psychological needs of the patient and the demands of the biomedical system.

(4) Patterns of fit and lack of fit between the needs and tasks of the individual and those of the family.

(5) The effects of the psychological meaning of juvenile diabetes on the health and social functioning of the patient and family. Possible effects include feelings of vulnerability, guilt, and stigmatism, use of denial, loss of control, and depression.

In addition to providing a model for the study of psychosocial and biological coping interactions, the research has direct clinical relevance as physicians need to base their own behavior as well as their comprehensive therapeutic interventions on this knowledge. Furthermore, the study will provide important new information about developmental issues in adolescence as well as provide new data for management of chronically ill patients who, despite current treatment approaches, continue to present major medical problems.

This project is being carried out in collaboration with Dr. Marvin Cornblath of the Neonatal and Pediatric Medicine Branch, NICHD. Dr. Cornblath and his associates shall make the biomedical assessments.

Data collection has begun.

Publications:

Hamburg, B.A. and Killilea, M.: Relation of Social Support, Stress, Illness and Use of Health Services. in: Selected Readings in Disease Prevention: Report of the Surgeon-General on Health Promotion and Disease Prevention, Part II. U.S. Department of Health, Education and Welfare, Public Health Service Publication. Washington, D.C., U.S. Government Printing Office, In press.

Hamburg, B.A.: Coping and Health: Early Adolescence as a Life Stress. In Levine, S. and Ursin, H. (eds.): Coping and Health: Proceedings of a NATO Conference. New York, Plenum Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00478-23 LN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
OTHER:	L. Ungerleider	NEI Staff Fellow	LN NIMH
	B. Turner	Professor	Howard U.

COOPERATING UNITS (if any)

Howard University

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	1.4	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate cortex is the source of two major, corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnecting the striate, prestriate, posterior parietal, and dorsal prefrontal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, inferior temporal and ventral limbic areas, and is critical instead for object vision. Our research concerns both pathways, but the accompanying report focuses on the second, or ventral one. Our studies suggest that each of the extrastriate links in this ventral pathway adds a different capability to object vision, making possible, in turn, highly efficient object perception, object memory, and object-affect associations. The sequential dependency of these behavioral functions is paralleled by the sequential dependency of the successive neural structures in the chain.

PROJECT DESCRIPTION:

The objective of the six sub-projects described here is to define the role in object vision of neural structures outside the primary visual projection system, using behavioral and anatomical analyses. (1) Monkeys were prepared with various lesions or injections of the lateral and medial striate cortex and the brain studied with the Fink-Heimer silver impregnation and autoradiographic methods for determining cortical projections. Results thus far have revealed three topographically organized representations of the visual field within the prestriate cortex. (2) Monkeys are being prepared with as complete ablations as possible of the striate-projection zone defined in (1) prior to training them on visual pattern discriminations. The results will provide a rigorous test of the hypothesis that the prestriate cortex serves as the link between the striate cortex and the inferior temporal areas. Concurrently, to determine whether even small prestriate lesions might "disconnect" inferior temporal cortex from corresponding parts of the visual field, monkeys with such lesions will be tested for discrimination ability in different fields with the aid of a fixation-monitoring device newly developed for us by the Section on Technical Development. (3) Previous ablation studies had demonstrated that stimulus equivalence between the visual half-fields depends on the convergence of their projections onto single inferior temporal neurons. Follow-up studies using the fixation-monitoring device will test whether inferior temporal neurons account for stimulus equivalence across any retinal translation, and also whether, as suspected, it is the neurons in the posterior part of inferior temporal cortex that are mainly responsible for this stimulus equivalence mechanism. (4) Monkeys with various cerebral lesions were compared on tests designed to measure one-trial visual memory for objects. Only removals within the anterior part of the inferior temporal cortex (area TE) impaired this object recognition ability, suggesting that this is the locus in which the visual trace left by previous viewing is preferentially established. By contrast, on tests designed to measure one-trial memory of object-reward associations, equivalent impairments were obtained after area TE lesions and after amygdalectomy. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. (5) In the process of investigating the role of temporal-lobe structures in memory we have chanced on a result that is particularly exciting because it may help to resolve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that if the hippocampus is removed in combination with the amygdala then a profound memory loss ensues, one that is even greater than that produced by lesions of inferior temporal cortex. Furthermore, the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings, it also provides a new insight into the neural mechanisms of memory. (6) The discoveries in (4) and (5) were antedated and guided by anatomical findings indicating that inferior temporal cortex projects both directly to the amygdala and indirectly to the hippocampus via the entorhinal area. To extend and refine our knowledge of these anatomical circuits, we investigated with both axonal degeneration and transport methods the inputs to the limbic structures of the temporal lobe. Results thus far indicate that no visual area caudal to area TE projects to the amygdala, but that all sections of area TE do, and that they do so within a circumscribed part of the basolateral nuclei. In addition, other sensory systems (i.e., olfactory, gustatory, somatosensory, and auditory) project to other parts of the amygdaloid complex in such a manner as to suggest that each sensory system has a dominant influence in a given part. These findings will help guide further behavioral studies into the possible mnemonic functions of the limbic system in all sensory modalities.

Scientific Significance to Mental Health Research. Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events which intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually-mediated behavior opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision is aiding greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

Publications:

Turner, B. H. and Mishkin, M. A reassessment of the direct projections of the olfactory bulb. Brain Research, 151: 375-380, 1978.

Turner, B. H., Mishkin, M. and Knapp, M. E. Distribution of the anterior commissure to the amygdaloid complex in the monkey. Brain Research, 162: 331-337, 1979.

Seacord, L., Gross, C. G. and Mishkin, M. Role of inferior temporal cortex in interhemispheric transfer. Brain Research, 167: 259-272, 1979.

Mishkin, M. Analogous neural models for tactual and visual learning. Neuropsychologia, 17: 139-151, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00480-15 LN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development and Plasticity of Primate Frontal Lobe

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Chief, Section on Developmental Neurobiology	LN	NIMH
Other:	R. M. Brown	Senior Staff Fellow		NIDA
	L. J. Porrino	Staff Fellow	LN	NIMH
	N. Bugbee	NIMH Extramural Fellow	LN	NIMH
	S. Kojima	Visiting Fellow	LN	NIMH

COOPERATING UNITS (if any)

National Institute of Drug Abuse, ADAMHA, DHEW

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Section on Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12.4	4.0	8.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of the Section on Developmental Neurobiology is the elucidation of basic principles governing the development and plasticity of prefrontal granular cortex in primates. The research program is composed of three integral parts: (1) analysis of the development and organization of certain classes of prefrontal cortical connections; (2) analysis of the functional maturation of the prefrontal cortex and related structures; and (3) assessment of structural and functional plasticity which follows early injury to the prefrontal cortex. The goals of this multifaceted project are achieved by a broad multidisciplinary approach including behavioral, anatomical, electrophysiological and biochemical techniques. These techniques are used in conjunction with recently refined procedures that permit prenatal neurosurgery on the primate brain with return of the fetus to the uterus and survival to and after term. The studies are conducted on the rhesus monkey because the expanse of association cortex in this species as well as its neuronal organization, protracted development and capacity for complex behavior makes it an excellent animal model for the study of normal and pathological development of human neocortex.

Organization and Development of Prefrontal Connections

A. Selective Association of Prefrontal Cortex with the Limbic System

Although one of the distinctive features claimed for prefrontal granular cortex is its intimate association with both cortical and subcortical components of the limbic system, information on the organization and topography of these connections is still not sufficiently detailed. Our anatomical analyses of prefrontal cortex in the primate brain have begun to provide new and important information about this significant class of prefrontal limbic connections, particularly those circuits which include the amygdala.

The projections of the amygdala to prefrontal association cortex in rhesus monkey were studied using horseradish peroxidase histochemistry and autoradiography for tracing neural connections. Microquantities of HRP were injected into one of the five following subdivisions which together comprised virtually the entire prefrontal association cortex: (1) the superior prefrontal gyrus on the dorsomedial wall of the hemisphere; (2) the dorsal bank of the principal sulcus and adjacent dorsal convexity; (3) the ventral bank of the principal sulcus; (4) the lateral inferior convexity; and (5) the medial orbital and rectus gyri. After two days, the monkeys were perfused with mixed aldehydes and frozen sections were reacted with benzidene dihydrochloride.

Following HRP injections in the superior prefrontal, medial orbital and rectus gyri, labeled neurons were located in the lateral and basolateral nuclei of the amygdala. However, no reaction product could be located in neurons in any part of the amygdala following HRP injections into the cortex of either the dorsal or ventral banks of the principal sulcus or the cortex of the lateral inferior convexity.

To expose the full extent of amygdalo-prefrontal projections directly, all nuclear components of the amygdala were injected with tritiated amino acids. In serial sections processed for autoradiography, radioactivity was distributed in sharply circumscribed territories of the prefrontal cortex. Supporting our HRP data, silver grains were found only over the orbital cortex and gyrus rectus on the ventral and ventromedial aspects of the lobe and also over the superior prefrontal gyrus on the medial surface of the hemisphere. In marked contrast, label did not exceed background levels over the territories of the entire dorsolateral convexity that extend from the edge of the hemisphere to the longitudinal fissure, including the principal sulcus.

Thus, on the basis of amygdalofugal projections, the primate prefrontal cortex of rhesus monkeys may be divided into two sharply segregated systems: i) a ventromedial system which constitutes the entire prefrontal projection of the amygdala and ii) a dorsolateral system which is essentially devoid of amygdalofugal projections. The ventromedial subdivision seems to correspond to the prefrontal cortex of the rat in which projections from the amygdala, the mediodorsal nucleus of the thalamus and the ventral tegmentum overlap. However, there appears to be no obvious homologue in the rodent comparable to the extensive amygdala-free dorsolateral convexity so prominent in primates.

B. Bilaterality of the Cortico-thalamic Pathway

It is generally considered that the mammalian neocortex projects exclusively to the ipsilateral thalamus. This view is based largely on studies using reduced silver impregnation methods for staining normal or degenerating fibers. Using recently developed autoradiographic techniques for tracing neuronal pathways, we observed a distinct bilateral distribution of cortico-thalamic terminals. Recognition that cortico-thalamic projections have a contralateral component may revise our concepts about the topographic organization of this important class of cortical efferents.

Eight infant and juvenile rhesus monkeys were injected with a mixture of ^{3}H-proline and ^{3}H-leucine into three selected cytoarchitectonic areas of the frontal lobe: the middle of the dorsal bank of the principal sulcus, the inferior prefrontal convexity, or the medial orbital gyrus. As expected, heavy concentrations of label were found in appropriate sites in the thalamus (e.g., the reticular, the ventral anterior and the mediodorsal nuclei) of the injected hemisphere in all animals. However, in addition to these well established ipsilateral projections, we also observed considerable radioactivity in certain areas of the thalamus of the opposite hemisphere. These crossed connections vary both in degree and localization. In accord with previous studies of ipsilateral thalamo-cortical pathways of the frontal lobe, contralateral projections from the cortex of the principal sulcus terminate primarily in the lateral parvocellular portion of the mediodorsal nucleus; contralateral fibers from the orbital cortex project to the magnocellular subdivision situated more medially in the nucleus. The inferior convexity of the frontal lobe issues its contralateral projections to cells lying intermediate to and overlapping both the parvocellular and magnocellular moieties of the mediodorsal nucleus.

Cortico-thalamic efferents have been recognized for their strategic importance in mediating cerebral cortical control over incoming sensory input that converges upon the dorsal thalamus. The present findings indicate that in the monkey the cortex of one hemisphere may exert such influence over the contralateral as well as the ipsilateral thalamus. It may be wondered whether similar bilateral cortico-thalamic representation exists in man, in whom some regions of each hemisphere have become dominant for specific functions. Bilateral cortico-thalamic projections could provide a mechanism for such regions situated in one hemisphere to exert control over thalamic input on both sides of the brain.

C. Prenatal Development of Prefrontal Connections

Our studies on the formation of prefrontal cortical connections have focused this year on the callosal pathways which interconnect the two hemispheres and on the cortico-striatal projections. We have previously shown that the terminals of both classes of connections display a highly organized modular distribution in the adult brain. In addition, we have discovered that the cortico-striatal pathway exhibits a remarkable degree of reorganization in response to brain injury. The purpose of the studies that we conducted in the past year was to determine i) the timing and sequence of outgrowth of these classes of connections; ii) the mode of their formation into modules; and iii) whether the cortico-striatal projection is distributed bilaterally at early stages of gestation and later becomes mainly ipsilateral. The latter possibility is one of several mechanisms that I proposed to account for the rearrangement of fronto-striatal connections that we found after unilateral resection of the dorsolateral prefrontal cortex at midgestation.

In order to analyze these questions, injections of a mixture of ^{3}H-proline and ^{3}H-leucine were made in the prospective dorsal bank of the principal sulcus in rhesus monkey fetuses at embryonic day 69 (E69), E95, and E155 (in the rhesus monkey full term lasts 165 days). Following these injections, the fetuses were replaced in utero and allowed to survive for several days. After this time, they were removed by a second Caesarean section and fixed by intracardial perfusion with mixed aldehydes, their brains were processed for light microscopic autoradiography.

Our preliminary findings indicate:

(1) The principal long-tract pathways of the prefrontal cortex are layed down well before birth in the rhesus monkey. This was suspected, of course, but not really known. The callosal pathway enters the homotopic cortex in the contralateral hemisphere between E95 and E155; the cortico-striatal pathway forms earlier between E69 and E95. However, further experiments in more closely spaced fetuses are necessary to determine the precise timing and sequence of innervation.

(2) Columnar organization of the callosal pathways is achieved at least two weeks before birth and perhaps somewhat earlier. This precocious appearance of modular organization in the connections of the frontal lobe contrasts with the postnatal formation of ocular dominance columns in the primary visual cortex of the same species.

The cortico-striatal pathway develops in at least two broad phases: in the first phase, axons from the prefrontal cortex are distributed uniformly throughout the caudate nucleus; in the second phase, these axons appear to become redistributed into an intricate pattern in which clusters of prefrontal fibers surround round or elliptically shaped cores that are devoid of axons from prefrontal cortex. This mode of formation of prefronto-striatal modules is similar to the pattern of genesis of retino-geniculate, retino-tectal and geniculo-cortical fibers in the primate visual system. The concordance of findings from studies of descending projections from frontal association cortex with those from studies of primary sensory systems suggests that the biphasic diffuse-to-sculptured scheme may represent a general principle governing the formation of all central nervous pathways.

Functional Development of Prefrontal Cortex and Related Structures

A. Electrophysiological Activity of Prefrontal Neurons

Previous research in this laboratory has provided evidence that specific regions of the primate prefrontal cortex develop their functions at different rates and according to different time-tables of maturation. In the last several years we have studied the maturation of function in the dorsolateral prefrontal cortex by detailed electrophysiological analysis of prefrontal unit activity at different stages of development. This year we have focused on the classification of neuronal subtypes within this same region with the ultimate goal of relating functional classes to the underlying laminar and modular organization of the prefrontal cortex.

The electrophysiological studies are performed on rhesus monkeys that have been trained to perform cognitive delayed-response tasks requiring the integrity of the prefrontal cortex as well as on a number of control tasks that do not. After the animals are well trained, chronic indwelling cylinders are implanted in their skulls over the dorsolateral prefrontal cortex. Following recovery from surgery, neuronal activity is recorded while the monkeys are performing one or another task.

Our principal findings this year pertain to the types of activity that are found in frontal granular cortex and their relationship to behavior. The major findings are: (1) firing changes occur most frequently during the early phases of the delay in a delayed response trial; (2) prefrontal neurons exhibit their greatest sensitivity to spatial location during the same early phases of the delay, i.e., a neuron may fire preferentially to a stimulus on the left (as compared to a stimulus on the right) or vice versa most often during the early delay; (3) differences between experimental and control tasks are sharpest when neuronal activity under the two conditions is contrasted during the early phase of the delay. These analyses confirm and extend previous findings that the prefrontal neurons are specialized for coding spatial-mnemonic information of a high order of complexity and that a critical process (encoding or registration) underlying this form of information processing occurs during the early phases of the temporal interval over which information must be retained.

B. Biochemical Analysis of Development of Monoamines in Prefrontal Cortex

Studies on the development of monoamine systems in major cortical and subcortical structures of the primate central nervous system continues to be a major focus of our activities this year. Over the course of this year we have added a number of additional animals and age-points to our previous analyses. However, our recent findings which employ the methods of cation exchange chromatography and fluorimetric assay confirm our previous results that were reported last year. Briefly, there are considerable differences in the time-tables of development of dopamine, norepinephrine and serotonin in different regions of the nervous system. We have identified three major periods of maturation which span a) a neonatal stage from birth through 8 months of age; b) a juvenile stage from 8 months to 2-3 years of age; and c) maturity which begins at 2-3 years and extends to 15 years and over, which in rhesus monkey can be considered the equivalent of old age in man. Since the changes in brain chemistry with age are quite complex, it will be convenient to describe the major findings for each putative transmitter separately.

Dopamine is found in high concentrations in the prefrontal cortex of newborn monkeys. However, the level and rates of synthesis of this amine decline over the neonatal period from birth through 8 months of age. In contrast, dopamine content and activity in motor cortex increase over the same period, reaching a peak by 8 months of age. The decline in prefrontal dopamine and the increase in precentral dopamine may possibly reflect a redistribution of dopaminergic activity within the frontal lobe over the neonatal period of postnatal development as well as maturation of control mechanisms for dopamine synthesis and regulation. Peak-levels of dopamine that are attained in motor cortex in the first eight months may be related to the increase in motor dexterity and fine coordination that begins to be evident at this age. Dopamine content and activity in prefrontal cortex increases from 8 months to 2-3 years of age followed by a marked decrease in aged monkeys. The increase in prefrontal dopamine at the stage of sexual maturation correlates with our independent evidence from studies of surgical and reversible lesions and from electrophysiological data which indicate that the dorsolateral prefrontal cortex reaches maturity for certain cognitive functions at this age. The decline in dopamine in old-aged animals (which is restricted only to prefrontal cortical dopamine) may be related to the decline in cognitive efficiency that sometimes occurs in old age. If so, reversal of such disorders may be possible with dopamine agonists.

In contrast to dopamine, norepinephrine exhibits a steady and graded increase with age in both levels and biosynthetic activity in all areas of the brain where it is present in measurable concentrations. It is noteworthy that norepinephrine which is present in peak concentrations in somatosensory cortex at all ages falls off sharply in the 15 year old monkeys. Norepinephrine content of other cortical and subcortical regions is only minimally affected in old age.

Serotonin is rather uniformly distributed throughout the neocortex at all ages but its metabolite, 5-HIAA, is present in higher concentrations in posterior regions of the neocortex. Developmentally, serotonin synthesis rates are low in parietal and visual cortex over the neonatal period from birth through 8 months. However, between 8 months and 2-3 years, there is a 75% increase in synthetic activity in these regions, thus indicating an increasing sertonergic influence of the functions of parietal and visual cortex with maturity.

While the functional role of monoamines in neocortical activity and in development of such activity needs to be elucidated, the dynamic and regionally specific changes in monoamine concentration and activity suggests a differential role for the different transmitters in each area of the cortex. Further, the functions mediated by each cortical area may well be modulated by a different neurotransmitter at each developmental time period. Further studies are required to decipher the meaning of these shifts in neurotransmitter balance over various stages of the life span and to explore their significance in pathological as well as normal behavioral development.

C. Seasonal Variation in Monoamines in Primate Hypothalamus

In the course of our developmental studies of monoamine systems we made the surprising discovery that serotonin levels and rates of utilization exhibit marked seasonal variation in the hypothalamus of rhesus monkeys. This finding confirms and extends recent results obtained in postmortem studies of human subjects in Sweden.

Using cation exchange chromatography and spectrophotofluorimetry, we found that serotonin concentration in hypothalamus is very high in summer and low in winter. Further, levels of 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophan accumulation rates after synthesis inhibition parallel the seasonal variations in 5-HT, thus indicating that utilization parallels synthesis. In addition there may be an interaction between the catecholamines and indoleamine systems; when dopamine catecholamine synthesis is high, serotonin synthesis is low and vice versa. Further studies are needed to fully assess the functional and biomedical significance of such biochemical rhythms in the primate diencephalon.

Structural and Functional Plasticity of Primate Telencephalon

A. Rearrangement of Connections Following Resection of Prefrontal Cortex

A major goal of our efforts this year was to extend information on the reorganization of the cortico-striatal pathway that occurs following resection of the dorsolateral prefrontal cortex in one hemisphere 6 weeks prior to birth. Specifically, we are examining: (1) whether there is a critical period for the induction of anomalous neuronal pathways in the primate telencephalon; (2) whether abnormal reorganization of connections is a property of the cortico-striatal pathways exclusively; and (3) the relationship between anatomical reorganization and recovery of function after cortical lesions.

To shed light on these issues, we have performed unilateral cortical resections in fetal monkeys at various stages of gestation and at different stages of postnatal life. In the case of prenatal surgery the fetus is exteriorized following a laparotomy and hysterotomy performed on a timed-pregnant rhesus monkey. Cortical tissue in the inferior bank of the primordial arcuate sulcus and in both banks of the developing principal sulcus and all cortex lying on the dorsal convexity superior to the principal sulcus is ablated. After the surgery is complete, the fetus is returned to the uterus and allowed to survive until term which occurs on average at 165 days of gestation. At term, the fetus is delivered by Caesarean section if delivery is not spontaneous.

Our experimental plan is to assess these monkeys subjected to unilateral cortical resection at various stages of pre- and postnatal development on a battery of behavioral tests. Such testing requires more than one year to complete per animal. Following the termination of behavioral assessment, the animals will receive injections of tritiated amino acids into the principal sulcus in the hemisphere opposite to that previously resected. Since the subjects for anatomical analysis are still being tested behaviorally, we have not completed the anatomical studies as yet. However, preliminary findings indicate (1) that we may anticipate alterations in a number of classes of prefrontal connections; (2) these alterations can occur following unilateral resection at various ages ranging from two months before birth until two months after birth; (3) the degree of axonal reorganization may be correlated with recovery of behavioral functions.

B. Neurological, Neuropathological and Behavioral Effects of Thiamine Deficiency in the Rhesus Monkey

Another example of functional plasticity of the primate central nervous system has come from our recent studies on the behavioral and anatomical consequences of thiamine deficiency in the adult rhesus monkey. Thiamine deficiency is thought to be an etiologic factor in Wernicke-Korsokoff Disease.

Seventeen adult male rhesus monkeys were divided into five groups: Thiamine Deficient One Bout Group (TD1), Thiamine Deficient Two Bout Group (TD2), Thiamine Deficient Four Bout Group (TD4), Pair-fed Control Group (PFC), and Normal Control Group (NC). The animals in groups TD1, TD2, and TD4 were subjected to one, two, or four periods of thiamine deficiency, respectively, alternating with thiamine administration and recovery periods. The three PFC animals were matched to an animal in each of the three experimental groups (TD1, TD2, TD4) and given the same quantity of experimental diet as that animal, but were supplemented with thiamine. NC animals received Purina Monkey Chow ad. lib. Following the deficiency periods, experimental and control animals were tested on four behavioral tasks; object reversal, place reversal, delayed response, and delayed nonmatching-to-sample. Results showed that, regardless of the number of periods of deprivation, thiamine deficiency produced neurological symptoms such as anorexia, lethargy, ataxia, and ophthalmoplegia similar to those found in Wernicke-Korsakoff Syndrome. The histopathological changes consisted of well circumscribed bilaterally symmetrical lesions located in the basal ganglia, the parafascicular nucleus of the thalamus, the periaqueductal gray matter of the midbrain, the pons, structures in the floor of the fourth ventrical, and the roof nuclei of the cerebellum. The severity of the damage and the topographical distribution of the lesions varied with the number of periods of deficiency. Lesions were not found in the mammillary bodies or the medial dorsal nucleus of the thalamus, structures that are thought to be important for the memory impairment in Korsakoff patients. Nevertheless, the thiamine deficient animals showed a selective impairment on the delayed nonmatching-to-sample task. This impairment was not related to the number of periods of deficiency, but rather to location of pathological lesions. Nevertheless, one to four periods of thiamine deficiency is not sufficient to produce all of the clinical, histopathological, and behavioral symptoms characteristic of Wernicke-Korsakoff Syndrome. Altogether the evidence suggests that more prolonged deficiency is necessary to elicit the full range of clinical symptoms of this disease.

PROPOSED COURSE OF PROJECT:

The Principal Investigator of this project, Dr. Patricia S. Goldman, is leaving the NIMH-IR Program on September 30, 1979. Dr. Goldman plans to continue this line of research in her new position as Professor of Neuroscience in the Section of Neuroanatomy at Yale University School of Medicine.

AWARDS:

1978 ADAMHA Administrator's Award for Meritorious Achievement

PUBLICATIONS:

Brown, R. M., Crane, A. and Goldman, P. S.: Regional distribution of monoamines in the cerebral cortex and subcortical structures of the rhesus monkey: concentrations and in vivo synthesis rates. Brain Research 168: 133-150, 1979.

Brown, R. M. and Goldman, P. S.: Developmental changes in catecholamine content and activity in circumscribed regions of the cerebral cortex in the rhesus monkey. In Usdin, E., Kopin, I. J. and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 815-817.

Brozoski, T. J., Brown, R. M., Ptak, J. and Goldman, P. S.: Dopamine in prefrontal cortex of rhesus monkeys: Evidence for a role in cognitive function. In Usdin, E., Kopin, I. J. and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 1681-1683.

Crane, A. and Goldman, P. S.: An improved albumin-gelatin method for embedding brain tissue. Stain Technology, in press.

Goldman, P. S.: Neuronal plasticity in primate telencephalon: anomalous crossed cortico-caudate projections induced by prenatal removal of frontal association cortex. Science, 202: 768-770, 1978.

Goldman, P. S.: Contralateral projections to the dorsal thalamus from frontal association cortex in the rhesus monkey. Brain Research 166: 166-171, 1979.

Goldman, P. S. and Galkin, T. W.: Prenatal removal of frontal association cortex in the rhesus monkey: anatomical and functional consequences in postnatal life. Brain Research 52: 451-485, 1978.

Goldman, P. S. and Rakic, P.: Impact of the outside world under the developing primate brain: perspective from neurobiology. Bulletin of the Menninger Clinic 43: 20-28, 1979.

Brozoski, T. J., Brown, R. M., Rosvold, H. E. and Goldman, P. S.: Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00481-07 LN

PERIOD COVERED

October 1, 1978 to September 30, 1979 (Incorporated into Z01 MH 00480-15 LN)

TITLE OF PROJECT (80 characters or less)

Pre- and post-natal genesis and plasticity of connections in the primate brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Chief, Section on Developmental Neurobiology	LN NIMH
OTHER:	D. Tanaka	Assistant Professor	Howard University

COOPERATING UNITS (if any)

Howard University

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02031-03 LN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in spatial perception

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
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	R. Nakamura	Research Psychologist	LN NIMH

COOPERATING UNITS (if any)

None

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	0.6	1.8

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate and prestriate cortex project not only ventrally, to the inferior temporal area, but also dorsally, to the posterior parietal and dorsal prefrontal areas. This dorsal projection pathway appears to constitute the visual contribution to a system for spatial perception. Many modalities undoubtedly contribute to this spatial system, which can be divided into a posterior (parietal) portion serving allocentric localization, and an anterior (prefrontal) portion serving autocentric localization. The entire system is probably critical for the spatial guidance of movement, and in keeping with this its major cortical outflow appears to be to the motor system. In addition, however, this spatial system appears to provide an essential tonic influence on the diffuse activating system, for its removal has been found to cause a profound sensory neglect.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The first two sub-projects described below deal with the parietal and prefrontal portions of the spatial system, respectively.

(1) Animals trained on a visual landmark test, a measure of allocentric localization ability, were prepared with posterior parietal removals in one hemisphere and complete striate removals in the other, and these were then followed by a callosal transection. Results indicate that the crossed lesions alone produced severe impairment and that, once the animal recovers, the addition of a collosal transection had only a mild effect. Thus, the posterior parietal cortex, like the inferior temporal, appears to depend heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy visual input via the corpus callosum. In a second experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal areas, the striate inputs to these regions appear to be organized differently, presumably reflecting differences in the cortical processing required for spatial vs object vision.

(2) A crossed-lesion experiment like that described above has shown that the dorsal prefrontal cortex also depends on striate input for its visual function, in this case autocentric localization as measured by the spatial delayed response test. Evidence that the function is selectively spatial comes from an experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests, including delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the principal sulcus relearned quickly, in sharp contrast to their permanent failure to relearn spatial memory tasks. The dimensions of the spatial deficit were examined in another experiment which compared the effects of selective prefrontal lesions on up-down delayed alternation as opposed to the customary left-right delayed alternation. In every case, lesions known to impair performance on left-right tasks (e.g., principalis removals, or inferior frontal ablations) had just as severe an effect on the performance of the up-down task, while removals known to have little effect on left-right alternation (e.g., periarcuate lesions) also had little effect on the up-down version. Apparently, the spatial impairment after principalis lesions is not limited to left-right confusion, but encompasses the vertical dimension as well.

(3) In another project (Neural mechanisms in vision) evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal "spatial" system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere) and then removed all the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Broadmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect. It appears from these results that the dorsal "spatial" system normally provides an activating input (relayed perhaps through the activating system) that is necessary for the visual system and perhaps other sensory systems to function properly, and that this is in addition to its contribution to spatial mechanisms. For when the animals eventually do recover their use of vision, they show permanent impairment in the ability to guide their body and limbs to visual targets; that is, they then show the expected spatial disorientation.

We have found, finally, that by extension of the lesion into the limbic system animals can be rendered chronically blind. Such preparations are now being used to test the loss-of-activation hypothesis directly by the method of unit recording in the intact visual system.

Significance to mental health. Spatial orientation in animals is an extremely well developed, higher order, perceptual mode. It is "higher order" in the sense that it depends not on a single sensory modality but on the coordinated functioning of several of them. Our evidence points to a dual cortical localization for this suprasensory modality in the posterior parietal and dorsal prefrontal areas. Analysis of this complex, multimodal, cognitive system in monkeys may help us to understand the organization of yet another complex, multimodal, cognitive system in man, namely, the one for language. The formal similarities between the language system (for which man's left hemisphere is specialized) and the spatial system (for which his right hemisphere is specialized) give added support to the validity of this approach.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02032-03 LN

PERIOD COVERED

October 1, 1978 to November 10, 1978 (Project has been temporarily suspended)

TITLE OF PROJECT (80 characters or less)

Neural Coding of Visual Stimuli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. D. Jarvis	Research Psychologist	LN NIMH
OTHER:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
	J. Levinson	Professor	University of Maryland

COOPERATING UNITS (if any)

University of Maryland

LAB/BRANCH

Laboratory of Neuropsychology

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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The results of our previous electrophysiological studies have shown that cells in the inferior temporal cortex seem more concerned with the physical properties of stimuli than with their motivational significance. In those studies we were unable to identify the stimulus parameters that seemed critical in determining the responses of the cells. Thus, a central question that remained unanswered is that of stimulus coding - to what characteristics of the visual world are cells in the IT cortex particularly sensitive. Recent studies have suggested that the visual system may be particularly sensitive to the spatial frequency components of and contrast within objects - i.e. the distances between and differences in brightness of light and dark areas. Our proposed study of the contrast sensitivity function of normal monkeys and those with selective lesions in the visual pathways is now almost fully instrumented. It is our hope that the results of this lesion study will suggest future directions for our electrophysiological investigations. The Principal Investigator resigned and the project has been temporarily suspended awaiting the arrival of her replacement.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02033-02 LN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Functional Mapping of Sensory Systems in the Primate

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
OTHER:	C. Kennedy	Guest Worker	LCM NIMH
	K. Macko	NIMH Extramural Fellow	LN NIMH
	E. Murray	NIMH Extramural Fellow	LN NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

NIMH, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	1.4	1.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects (i.e. object vision) extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal areas, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe. We have attempted to map this functional system by means of the 2-deoxyglucose technique, an autoradiographic method for measuring local cerebral glucose utilization (LCGU). To achieve this, we compared LCGU values in a surgically "blinded" hemisphere and in the opposite "seeing" hemisphere in the same monkey while the animal was visually active. Cortically, we found reduced glucose utilization in the blind as compared to the seeing hemisphere not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed areas also included tissue thought to be important in spatial, rather than object, vision.

Subcortically, side-to-side differences were seen in structures associated with processing information about both visual objects and visual space. The results not only provide striking confirmation of an extensive cortical visual system but also help to reveal its full extent and to localize its points of contact with subcortical structures.

Project Description

Neural structures involved in the processing of visual information have been painstakingly identified through the use of very time-consuming surgical electrophysiological, and anatomical techniques, and yet, the full extent of the visual pathway has probably not been revealed. These techniques have been used to describe a sequential system for processing information about visual objects which begins in the geniculostriate pathway and continues through the prestriate cortex along a corticocortical route to the inferior temporal cortex. Information is then transmitted to limbic and striatal structures, although the precise termination of this system in those areas is unclear. These techniques have also been used to identify another system, specialized to process information about spatial vision, which also depends on a geniculostriate input and a corticocortical pathway, in this case through the prestriate, parietal and prefrontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway.

We have attempted to map these functional systems by means of the 2-deoxyglucose technique, an autoradiographic method for measuring cerebral glucose utilization (Kennedy et al., Science, 187: 850, 1975). To achieve this, we prepared monkeys with a unilateral optic tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare values for local cerebral glucose utilization in a "seeing" and "blind" hemisphere within the same animal, and thereby map the visually related areas.

We also prepared monkeys with an optic tract section alone. In these monkeys, any information coming into the "blind" hemisphere would be received by way of corpus callosum and anterior commissure. Optical densities would then reveal the pattern of these interhemispheric connections.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively-labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively-labeled substance is incorporated into active cells during this period and any extracellular deoxyglucose is of low enough concentration so that its presence does not contaminate the autoradiographs.

In a second study, monkeys with both an optic tract section and split of the forebrain commissures were trained on a visual discrimination task using the hand opposite the blind hemisphere. The task was a pattern discrimination in which a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials. Correct responses to the positive were reinforced with a liquid reward. No reward followed correct responses to the negative stimulus.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system and its subcortical projections, asymmetries were also seen in the superior colliculus, inferior and lateral divisions of the pulvinar, and lower bank of the intraparietal sulcus and exposed surface of the inferior parietal lobule. Performance on the discrimination task led to a symmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking; within the visually related areas, however, the effects of the two different-testing situations were substantially the same.

The results of these studies in the visual system have provided in a short period of time such striking confirmation of the extent and the locus of visually related areas that we are now undertaking similar experiments involving other sensory systems for which (unlike vision) the central processing pathways are still virtually unknown. We are also conducting studies on vision in infant monkeys in order to trace the functional development of this sensory system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02034-01 LN

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Subcortical Mechanisms Related to Frontal Lobe Functions in the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. E. Rosvold	Chief, Laboratory of Neuropsychology	LN NIMH
Other:	T. J. Brozoski	Professor	Grinnell College

COOPERATING UNITS (if any)

Grinnell College, Grinnell, Iowa

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.6	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The intimate anatomical and biochemical relationship between the caudate nucleus and the substantia nigra suggests that they may subsume similar cognitive functions. It has been established that lesions placed in the caudate nucleus of the rhesus monkey impair performance on delayed alternation performance. However, massive lesions placed in the substantia nigra of a pilot animal had little, if any, effect. It becomes important therefore to establish the generality of this negative finding, and to explore what significance for behavior there is in the biochemical and anatomical similarities in these structures.

PROJECT DESCRIPTION

The close relationship between the caudate nucleus and the substantia nigra has been demonstrated both anatomically and biochemically. It would be expected, therefore, that these two structures would subsume similar cognitive behavior, yet it has not been shown that they do. The purpose of this project is to examine the possibility that lesions placed in the substantia nigra would, as they do in the caudate nucleus, result in severely impaired performance on the delayed alternation task, a test of cognitive behavior.

Stereotaxic procedures will be used to place the lesions in the substantia nigra of the rhesus monkey with the intention of destroying most of the structure bilaterally. The animals will then be tested on a battery of tests known to reflect impaired function in the caudate nucleus. When the tests have been completed, the brain will be removed and the site of the lesions verified histologically. Although it is difficult to place lesions selectively in the substantia nigra, we have demonstrated in a pilot animal that it is possible to do so. This animal, was, surprisingly, not impaired in performance of the delayed alternation task. It becomes extremely important, therefore, to establish the reliability of this negative finding. Four animals have been prepared on the behavioral tasks and are ready to be operated.

Significance to Mental Health. The monoamines, chemical products of the substantia nigra, raphe nuclei, and the locus ceruleus, have been implicated in mental health and disease. It is not known to what extent they also affect cognitive behavior. It is the purpose of this project to provide this information which presumably could be of diagnostic or therapeutic use.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00471-24 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of Heredity and Environment in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Rosenthal	Chief, Section on Genetics of Mental Illness	LPP NIMH
OTHER:	P. H. Wender	Professor of Psychiatry	University of Utah
	S. S. Kety	Professor of Psychiatry	Harvard Medical School
	S. Nagler	Research Psychologist	Institute for Research on Kibbutz Education (Israel)
	F. Schulsinger	Co-Director	Psychological Institute (Denmark)
	O. W. Quinn	Guest Worker	LPP NIMH

COOPERATING UNITS (if any)

Harvard Medical School, Institute for Research on Kibbutz Education, and Psychological Institute

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
Section on Genetics of Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.1	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

PHS-6040
(Rev. 10-76)

Project Description:

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders and a modified version of this book have been published. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation, and eventually a monograph will be written on the adoption studies. Data analysis is continuing. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Four subjects have had a breakdown, and all have a schizophrenic parent.

It is significant to bio-medical research and the program of the Institute that we have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

The proposed course of our project is to examine additional subjects in Denmark with the following goals in mind: (1) to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; (2) to compare the fate of gene carriers who are reared in the parental home and compared to those reared in adoptive homes; (3) to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; (4) to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we are carrying out reexaminations of this unique sample of subjects.

Publications:

Rosenthal, D.: Discussion of J. Zubin's "Abnormal psychology - Use and misuse of the biometric approach." In Salzinger, K. and Denmark, F.L. (Eds.): Psychology: The State of the Art. New York, The New York Academy of the Sciences, 1978, vol. 309, pp. 114-116.

Rosenthal, D.: Eugen Bleuler's thoughts and views about heredity in schizophrenia. Schizophrenia Bulletin 4: U.S. DHEW, PHS, ADAMHA, NIMH. DHEW Publ. No. (ADM) 78-145. Washington, D.C., U.S. Govt. Print. Off., 476-477, 1978.

Rosenthal, D.: Was Thomas Wolfe a borderline? Schizophrenia Bulletin 5: U.S. DHEW, PHS, ADAMHA, NIMH. DHEW Publ. No. (ADM) 79-145. Washington, D.C., U.S. Govt. Print. Off., 87-94, 1979.

Schulsinger, F., Kety, S.S., Rosenthal, D., and Wender, P.H.: A Family Study of Suicide. In Schou, M. and Strömgren, E. (Eds.): Origin, Prevention and Treatment of Affective Disorders. London, Academic Press, 1979, pp. 277-287.

In press:

Rosenthal, D.: Foreword. In Shakow, D.: Adaptation in Schizophrenia: The Theory of Segmental Set. New York, John Wiley and Sons, in press.

Rosenthal, D.: Genetic Aspects of Schizophrenia. In van Praag, H.M., Lader, M.H., Rafaelsen, O.F., and Sachar, E.J. (Eds.): Handbook of Biological Psychiatry, Volume II, Brain Mechanisms and Abnormal Behavior. New York, Marcel Dekker, Inc., in press.

Rosenthal, D.: Genetic Factors in Behavioral Disorders. In Roth, M. (Ed.): Psychiatry, Genetics and Pathology: A Tribute to Eliot Slater, London, Headley Brothers, Ltd., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00472-16 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Investigations of Some Formal Characteristics of Speech

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	D.S. Boomer	Guest Worker	LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Psychology & Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigates attention during natural speech perception. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of reaction time to irrelevant stimuli--clicks--while the subject is listening to a tape recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support this method will be used to investigate attention deficit in schizophrenia.

PHS-6040
(Rev. 10-76)

Project Description:

An extended psycholinguistic research program, detailed in previous annual reports, has contributed to a developing theory of speech production and perception, original in this laboratory. The central hypothesis is that the phonemic clause, a linguistically defined unit of speech averaging about 7 or 8 syllables, is a strategic functional unit in both the production and perception of speech.

During the past year the present research program has focused on speech perception. The guiding hypothesis, growing out of work on listener responses, is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units then attention to the incoming speech need not be continuous, even though the speech itself is continuous. Rather than attending to fine detail in the stream of speech, syllable by syllable and word by word, the listener can accumulate a series of 7 or 8 syllables in short-term memory and at its conclusion decode it as a pattern of sound and meaning. If speech perception in fact operates in this way one would expect that the listener's attention would fluctuate, being maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

In the present study subjects listen to a rather absorbing tape recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses; the other half occur within phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference will be reflected in the mean response latency to the two sets of linguistically-specified clicks.

Results show that for right-handed subjects, when speech is presented in the right ear and clicks in the left, reaction times were about 20 msec. faster to clicks at the terminal juncture than to those during the clause (18 of 20 subjects showed this effect). The data suggest that fluctuations of attention do occur during speech perception, but contrary to the original hypothesis speech processing seems to be more active during a clause than at the terminal juncture.

Currently, we are testing the generality of this result by testing a group of left-handed subjects and a group of right handers with speech in the left ear and clicks in the right. Laterality is being assessed by means of a questionnaire, behavioral tests and a dichotic listening procedure. Preliminary data suggest somewhat weaker effects in these groups but trends in the same direction as the previous finding.

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attention to the specification of hemispheric asymmetry in cortical function. It is widely believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00476-08 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Offspring of Schizophrenics: Markers of a Schizophrenic Disposition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D. Rosenthal	Research Psychologist	LPP, NIMH
	S. Broman	Research Psychologist	DNB, NINCDS
	P. Nichols	Research Psychologist	DNB, NINCDS
	P.H. Wender	Professor of Psychiatry	Univ. of Utah

COOPERATING UNITS (if any)
Developmental Neurology Branch, NINCDS

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
-

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of children of schizophrenics is based on data from an NINCDS study of the relationship between pregnancy and delivery conditions to include psychological and neurological functioning of the offspring, studied until age 8. We have found that: (1) There were more fetal deaths among the offspring of schizophrenics. (2) The IQ scores of the surviving offspring of schizophrenics appear to be more related to adverse perinatal factors than do IQ scores for the controls. (3) The behavior of the surviving male offspring of schizophrenics was more often rated as hyperactive or immature at age 7 than was the behavior of controls. These offspring also had more soft neurological signs, especially abnormal hand-arm coordination.

Project Description:

The project is aimed at discovering differences between the offspring of schizophrenics and controls, at an early stage in life, which may represent a predisposition to develop schizophrenia later in life. It is also aimed at evaluating the effect of perinatal complications on the children of schizophrenics. This study has utilized the data previously collected by the National Institute of Neurological and Communicative Disorders and Stroke, Perinatal Research Branch, in their "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood." In that project, some 55,000 pregnancies and the offspring were studied, with the 8-year follow-up now just completed. About 900 of the mothers or fathers had had a psychiatric hospitalization, and of this group, we took 220 who were studied in the Boston area to use for our study. We gathered the records of their psychiatric hospitalizations, and we proceeded to diagnose these subjects. We diagnosed 150 parents, and assigned a diagnosis of schizophrenia to 67 of these. The 150 families produced a total of 210 children who had been part of the NINCDS study, and 93 of these were the offspring of schizophrenics. Taking the families containing a schizophrenic parent, we selected a matched control group, and began comparing the findings for our two groups on the investigations which the NINCDS/PRB had performed.

We have found that there is an increased rate of fetal and neonatal death among the offspring of schizophrenics as compared with controls. Some of these deaths were associated with neurological malformations. Reasons for the deaths were not found at autopsy. The deaths were among the offspring of parents with chronic and severe forms of schizophrenia, as opposed to the acute or borderline forms. The deaths occurred predominately among those offspring who had two schizophrenic parents, or had a schizophrenic mother, rather than among those who had a schizophrenic father only. Two comparison groups, offspring of possible schizophrenics and offspring of nonschizophrenic patients, did not show an increased rate of fetal or neonatal deaths when compared to their matched controls.

There is a slightly lower IQ among the offspring of parents with the more chronic forms of schizophrenia when compared with controls at 7 years. Perinatal factors, such as edema, vaginal bleeding, spinal anesthesia at delivery and Apgar Score are quite highly correlated (in a negative direction) with IQ in this sample, whereas they are not highly correlated among the control population. These negative correlations were present whether the father or the mother was the schizophrenic parent, and thus the findings might reflect a genetic susceptibility of the infant to perinatal complications. By means of a multiple regression analysis it was discovered that these perinatal factor-IQ correlations could not be accounted for by demographic variables such as SES, maternal age, parity, etc.

Certain behavioral abnormalities, (hyperactivity, impulsiveness, distractability) were found among the male offspring of parents with the more chronic forms of schizophrenia. We also found a certain combination of abnormalities (hyperactivity and neurological signs), more frequently in these cases than among the controls. It does not necessarily follow that these behavioral disorders are genetically determined, but it is quite possible that the children with these abnormalities are the children who will later become schizophrenic, since hyperactivity is frequently found in the childhood histories of adult male schizophrenics. The perinatal factors which correlated with the offsprings' IQ were not found to correlate highly with these behavioral abnormalities.

The significance of this work to bio-medical research and the program of the Institute is that it adds to our knowledge of the etiology of schizophrenia. We need to find how the genetic factor underlying schizophrenia manifests itself if we are to understand the development of schizophrenia or to effectively intervene.

Publications:

Rieder, R.O., Nichols, P.L. The offspring of schizophrenics III. Archives of General Psychiatry 36:665-674, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00484-19 LPP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Psychophysiological Responsivity and Behavior in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	C. Schooler	Research Psychologist	LSES, NIMH
	M. Buchsbaum	Research Psychiatrist	APB, NIMH
	D. Murphy	Research Psychiatrist	CNB, NIMH
	D. Van Kammen	Research Psychiatrist	BPB, NIMH
	C.R. Lake	Research Psychiatrist	LCS, NIMH
	T.N. Robinson	Staff Fellow	LPP, NIMH
	R. Haier	Guest Worker	LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-environmental Studies, Biological Psychiatry Branch, Laboratory of Clinical Science, and Ward 4 East

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

General purpose: To investigate schizophrenic-normal differences in autonomic nervous system activity in various laboratory situations which differ in the demands placed on the subject, the kinds of stimuli presented and the type of behavior required. To determine biological and psychological processes related to ANS activity.

Peripheral measures of autonomic functioning: Skin conductance, heart rate, finger pulse volume, respiration and skin temperature are recorded continuously during periods of resting, presentation of mild tones and task performance. The tasks used include reaction time, mental arithmetic, two-flash threshold, and tachistoscopic recognition.

We showed that drug free acute schizophrenics who did not improve during a subsequent 4 month hospitalization were more deviant in ANS functioning at admission than were patients who improved. Current studies aim to replicate and extend this finding in patients who are on controlled drug trials to see if on or off drug ANS activity is related to therapeutic outcome.

PHS-6040
(Rev. 10-76)

Project Description:

In a completed study we showed that a pattern of ANS and attentional functioning demonstrated previously in chronic schizophrenics, also tended to be characteristic of acute schizophrenics who were selected for reasonably good prior functioning. Medication free schizophrenics tend to have higher ANS "arousal" levels (heart rate, frequency of spontaneous skin conductance responses), smaller ANS responses particularly to meaningful or demanding stimuli and situations, slow adaptation and habituation, and poor attention. Further, this pattern predicted the outcome of the NIH hospitalization (about 4 months). Patients with poor outcome strongly showed this pattern when tested shortly after admission to the unit, while patients whose psychosis later remitted, particularly males, were less deviant from controls. Good prognosis females were not as clearly distinguishable, particularly on the ANS response variables. Nevertheless, a discriminant analysis correctly classified 86% of the patients of both genders. Global psychopathology ratings and premorbid social functioning did not predict outcome.

On a test done shortly before discharge ANS and attentional functioning was very significantly related to remission status but, in general, not much different from that at admission. The dissociation between ANS activity and clinical state at admission and its changes over time suggest either that ANS activity is "trait" related or that its "normalization" in good outcome patients precedes clinical improvement.

Most patients were assessed 9-12 months after NIH discharge. Preliminary results show that ANS activity at admission is not closely related to follow-up status but that patients with a better clinical course had less deviant ANS functioning at discharge.

Data collection has been completed on another major study of a more heterogeneous group of medication free schizophrenic inpatients and normal controls. The protocol involves ANS recording during two sessions which include rest and stress periods, simple tones and two perceptual-motor tasks: two-flash discrimination and a tachistoscopic recognition procedure. We can test the differential psychophysiological responsiveness of schizophrenics and normals to task difficulty, success and failure, positive and negative reinforcements, etc., as well as being able to separate sensory reception aspects from response aspects of the stimulus complex. In addition to attempting to replicate and extend the previous findings on the relation of ANS activity to improvement we can also relate the ANS variables to the effects of different drugs such as lithium, pimozide, propanalol, GHB, and naloxone and the effects of renal dialysis which are being tried along with placebo on a double-blind basis on these patients. Thus, we can test the feasibility of using ANS variables to aid in the determination of the optimal therapy. In addition, we are testing patients while they are on the drug protocol using a procedure in which ANS variables are recorded during rest, presentation of a series of tones of different intensities and a two-flash threshold determination. These observations may help us determine how ANS changes relate to changes in behavior and to increase our understanding of the central mechanisms involved in of ANS functioning.

In normal controls tested on these same procedures we are investigating relationships between ANS activity, personality, behavior and enzyme activity. These subjects (N=97) were given personality, interest, and mood questionnaires and had platelet and plasma monoamine oxidase activity measured. Consistent results have been obtained when the large number of psychophysiological variables has been reduced by factor analysis. Scores on factors representing skin conductance responsivity were found to correlate positively with platelet MAO activity for both sexes but most consistently for women. Less consistent positive relationships were found between MAO activity and arousal factors. These data suggest, rather surprisingly, a positive relationship between sympathetic nervous system activity and MAO activity. Similar relationships were found in the study on acute schizophrenics, but just at the discharge test. Other data suggest a negative relationship between MAO activity and parasympathetic activity. Preliminary analyses show that ANS activity is related to low stimulus seeking in women, but to active life style in man. In collaboration with Dr. Schooler of LSES, the method of "confirmatory factor analysis," developed for use in social science research, is being used on the psychophysiological and personality data in an attempt to develop error free measurement models of the structures of these systems. This method may lead to causal models of the interrelationships between systems. Similar studies emphasizing autonomic orienting responses to tones of different intensities and using an overlapping battery of personality scales are in progress in collaboration with Dr. T. Robinson.

Investigations of the relationships between the ANS response to stress and changes in plasma catecholamines have been done in collaboration with LCS and the NHLBI. Normal controls, hypertensive patients and orthostatic hypotensive patients were given 3 psychological stress procedures (having a balloon popped, mental arithmetic, and electric shocks) and 5 minutes of standing up interspersed with rest periods while skin conductance and heart rate were recorded continuously, blood pressure taken frequently, and periodic blood samples were drawn. These will be analyzed for norepinephrine. Prelimary analyses show hypertensives, who were off medication, were quite low in ANS response to stress. A similar study has been completed on 20 middle-aged normal males thought either to have a "coronary prone personality" (Type A) or not (Type B). These subjects were given a mental arithmetic stress procedure and the "Type A, B" interview as well as the 5 minute standing procedure. Plasma norepinephrine shows remarkably little increase during psychological stress despite marked autonomic activation, but the expected increase in both sets of measures during standing was found. We hope this study will help elucidate the mechanisms involved in response to stress, will lead to improved methodology for future studies, and will give us some notion of how individual differences in personality relate to physiological responses to stress.

New studies of attentional processes in schiozphrenia have been begun. Several methods of assessing attention deficits using reaction time (RT) techniques are being compared: 1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm.

2) a choice RT procedure to investigate "sensory dominance." In this method RT to visual and auditory stimuli is measured when the stimuli are predictable, unpredictable or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance. 3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending to.

These procedures have been used in a "high risk" study in collaboration with Dr. Buchsbaum of BPB in which S's were selected for extremely good or poor performance on another test of attention: the continuous performance task. ANS data were also recorded during the "set" procedure. A variety of cognitive, perceptual and personality data were gathered on these subjects in order to assess possible relationships to psychopathology.

These procedures are also being used on medication-free schizophrenic inpatients on a BPB clinical unit. Several theoretical models about attention dysfunction in schizophrenia can be tested. Most patients tested have shown visual dominance and intersensory facilitation but more data are needed for quantitative comparisons.

Publications:

Zahn, T.P., and Carpenter, W.T.: Effects of short term outcome and clinical improvement on reaction time in acute schizophrenia. J. Psychiatric Res.. 14:59-68, 1978.

Zahn, T.P. Sensitivity of measurement and electrodermal "nonresponding" in schizophrenic and normal subjects. Schizophrenia Bulletin 4:153, U.S. DHEW, PHS, ADAMHA, NIMH, DHEW Publ. No. (ADM) 78-145. Washington, D.C., U.S. Gov't Print. Off., 1978.

Zahn, T.P. The reality of the PPI-PI effect. Schizophrenia Bulletin 4:156, U.S. DHEW, PHS, ADAMHA, NIMH, DHEW Publ. No. (ADM) 78-145. Washington, D.C., U.S. Gov't Print. Off., 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00486-07 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Psychophysiological Concomitants of Minimal Brain Dysfunction in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	J.L. Rapoport	Medical Officer	BPB, NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators believe that the autonomic nervous system (ANS) may be involved in hyperactivity (HA) in children. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators such as skin conductance, heart rate and skin temperature, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on attention such as reaction time. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of d-amphetamine on autonomic activity and attention in 6-13 year-old normal and HA boys. Male adults have been tested on the same protocol to test for age differences in drug effects.

Project Description:

In a study done with Dr. J.L. Rapoport of BP, (Z01 MH 00151-01 BP) 6-13 year old normal boys and boys with a diagnosis of HA had the previously mentioned indices of ANS activity measured continuously during a session which included a rest period, presentation of a series of 75 dB tones and a test of attention (reaction time) in a drug-free baseline session and after being administered either placebo or d-amphetamine (.5 mg/kg) in a double-blind crossover study. The procedures used are very similar to those used in a previous study with larger samples of HA and normal children. The purpose of this study is to test the widely held hypothesis that HA children give paradoxical or atypical responses to stimulant drugs.

Results show similar effects of d-amphetamine in HA and normal children in terms of reduced motor activity, and faster reaction time. The drug also produced greater anticipatory heart rate deceleration, a response which is thought to reflect focussed attention, in both groups. Reduced electrodermal activity was found in both groups, but this was more pronounced to different aspects of the test procedure in the two groups. It was more marked for the normal group in responses to the reaction time stimuli, and in the HA children in responses to simple tones and in spontaneous electrodermal activity. In general, results show that the "paradoxical" quieting and attention-improving effects of stimulant drugs are not specific to hyperactive children, and this conclusion generalizes to most psychophysiological effects. The difference in patterning of the drug effects on skin conductance responsivity is puzzling. It suggests the changes in behavior may involve different mechanisms in the two groups. Planned correlational analyses may help solve the puzzle.

Comparison of the placebo condition with the baseline session produced some interesting results. On placebo, both groups showed significantly higher ANS "arousal," but also had an increase in motor activity and no change in attention. The interpretation of these findings is open to question, but they do disconfirm the hypothesis that the effectiveness of stimulant drugs in quieting HA children is due to their increase in ANS "arousal." Consequently, these data are also inconsistent with the widely held low arousal hypothesis of hyperactivity.

Normal adult males have been tested on the same protocol on both .5 mg/kg and .25 mg/kg d-amphetamine. Preliminary analyses of the results show that only the low dose group decreased in motor activity on the drug. This group also had faster RT under one of the conditions (long foreperiod, which is more demanding of attention). Only the high dose group had a consistent increase in "arousal" measures. This confirms the dissociation between arousal and behavior found with the children. In contrast to the children, d-amphetamine led to smaller heart rate deceleration in both adult groups, but like the children the drug tended to reduce the responsivity of the skin conductance system. Thus, the adults showed a moderately different response to d-amphetamine than the children, but, in general, there is little in this study to support the hypothesis of an atypical response to stimulant drugs in HA children.

We have begun a similar study to test the hypothesis that caffeine may potentiate the action of d-amphetamine.

Publications:

Zahn, T.P., Rapoport, J.L., and Thompson, C.L. Autonomic and behavioral effects of dextroamphetamine and placebo in hyperactive and normal prepubertal boys. J. Abnorm. Child Psychol., in press.

Rapoport, J.L., Buchsbaum, M.S., Weingartner, H., Zahn, T.P., Ludlow, C., Mikkelsen, E.J., Langer, D., and Bunney, W.E., Jr. Dextroamphetamine: Cognitive and behavioral effects in normal and hyperactive children and normal adults. Arch. Gen. Psychiat., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00487-07 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Autonomic Functioning in MZ and DZ Twins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	M. Buchsbaum	Research Psychiatrist	BPB, NIMH
	A. Sostek	Post Doctoral Fellow	BPB, NIMH

COOPERATING UNITS (if any)
Adult Psychiatry Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine the relative importance of genetic vs. environmental factors in various aspects of autonomic functioning and to determine the relationships of autonomic nervous system activity to personality, task performance and electrocortical functioning.

Peripheral measures of autonomic functioning, namely skin resistance, heart rate, finger pulse volume, respiration, and skin temperature are recorded during two sessions which include rest periods, a series of mild tones, a reaction time task and mental arithmetic. The subjects are 34 pairs of monozygotic twins and 34 pairs of dizygotic twins, about equally male and female. Some opposite sex DZ pairs have also been tested.

Project Description:

No further work on the genetic aspects of these data was done last year but in collaboration with Drs. Sostek and Buchsbaum of BPB the data are being reduced by factor analysis and interrelationships with electrocortical data on the same subjects will be examined. Such data reduction may also give us a clearer picture of the genetic determination of ANS activity.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00488-14 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Individual Differences in Survival and Reproduction Among Old Colony Mennonites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Allen	Guest Worker	LPP NIMH
OTHER:	C. W. Redekop	Professor of Sociology and Anthropology	Univ. of Waterloo and Conrad Grebel College

COOPERATING UNITS (if any)

University of Waterloo and Conrad Grebel College

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was undertaken to seek evidence of natural selection with respect to human behavioral characteristics. The population chosen consisted of about 13,000 German-speaking Mennonites whose culture resembles in many respects that of pre-industrial European peasants.

A census-survey was made in 1967, and vital records, kept by the church, were copied. The editing of these two types of record has been very time-consuming and is still in progress.

When editing is completed, relations will be explored between vital events and socio-economic variables. The principal hypothesis to be tested is that, under conditions of natural human reproduction, individual behavior that is economically successful results also in superior biological reproduction.

Project Description:

This investigation was undertaken in 1965, mainly to seek evidence of natural selection with respect to human behavioral characteristics. The study of selection in a pre-industrial type of European culture may indicate some of the influences that have produced present genetic tendencies and would draw a base line for future studies of selection under modern conditions. A secondary objective was to define natural patterns of marriage and reproduction that might have a bearing on the growth and regulation of human populations.

The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of 46 villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Professor Calvin Redekop, of the Department of Sociology and Anthropology at Conrad Grebel College and the University of Waterloo, has assisted in the planning and execution of the study. He speaks the Low German dialect of these people and has lived among them.

Information was gathered by means of a combined census and survey that reached a third of the population. This was supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals.

Editing of the data for accuracy and consistency was the most time-consuming part of the project, because each set of records, even our own census, was collected and recorded by poorly-educated farmers. After editing, the main data sets were subjected to cross-tabulation for general descriptive purposes. The survey data were then analyzed by correlational and factoring methods to yield ratings for each farmer in several dimensions of social and economic behavior. Comparison between the census and church records revealed many disagreements of types which can be reconciled, with resulting enhanced accuracy. This, however, necessitates laborious cross-editing between the two data sets.

The final phase of the study analyzes the relations between vital events and socio-economic variables and attempts to define family characteristics of people who subsequently have large vs. small families. This should be completed in two years, and another year may be needed for preparation of reports.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00489-22 LPP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Variables Affecting Twin Birth Frequencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Allen Guest Worker LPP NIMH

COOPERATING UNITS (if any)

Perinatal Research Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is temporarily inactive.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00491-03 LPP

PERIOD COVERED October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Personality factors and psychophysiological responses to changing stimulus input.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. N. Robinson, Jr.	Staff Fellow	LPP NIMH
Other:	T. P. Zahn	Research Psychologist	LPP NIMH
	R. O. Rieder	Research Psychiatrist	LPP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to shed information on how such factors as stimulus intensity, unchanged stimulus input, heteromodal stimulation, and sensory threshold task performance differentially affect autonomic nervous system (ANS) functioning in selected personality groups. Examined also is the relative correspondence of bilateral electrodermal activity to perceptual task performance. The relation of psychiatric diagnosis to electrodermal responsivity is examined by recalling a selected group of previously-tested subjects and providing them with a psychiatric interview. Black and white males are sampled in an effort to provide data on racial differences in ANS functioning to imperative and uncontrolled stimulus input.

PHS-6040
(Rev. 10-76)

Project Description:

The objectives of this project are to provide further clarification of the relationship of psychometric personality factors and psychophysiological response in normals and to assess the extent to which racial groups show ANS response stereotypy.

Data collection for each subject comprises two sessions separated by at least a week. Each session involves approximately 2-1/2 hours of testing. One-hundred-eighty volunteers have been tested to this point.

To date, selected findings indicate that personality differentially affects the pattern of electrodermal arousal under mildly stressful stimulation conditions. Significant differences in auditory threshold performance have been demonstrated also for a number of personality measures. Moreover, a psychometric measure of psychoticism has been shown to present results for normals scoring high and low on the scale in two-flash fusion and auditory threshold task situations that parallel those reported for shizophrenics and normals.

This project is significant to biomedical research and the program of the Institute in that a greater clarification of ANS functioning in normals will make more meaningful the comparison of normals and psychiatric patients in future psychiatric research. Moreover, because personality structure may play an important role in the expression of psychopathological illness, it is possible that a determination of the patterns of psychophysiological response associated with personality factors may help account for the large variability in ANS response noted among psychiatric patients. The determination of differential ANS response patterns for racial groups has implications for the genetics of psychophysiology and, as such, for the use of these techniques in discriminating aspects of the genetical component of mental illness.

The proposed course of the project is to continue testing until an additional 12 black male volunteers have been tested.

Publications:

Robinson, T.N., Jr., and Zahn, T.P.: Extraversion and neuroticism effects on EDR responsivity to variable intensity stimulation. Psychophysiology, 16(2): 182, 1979.

Robinson, T.N., Jr., and Zahn, T.P.: Covariation of two-flash threshold and autonomic arousal for high and low scorers on a measure of psychoticism. Br J Soc Clin Psychol, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00492-03 LPP

PERIOD COVERED October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relationships among personality, biochemical and psychophysiological variables in college students.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP	NIMH
Other:	M. S. Buchsbaum	Chief, Psychophysiology Section	BPB	NIMH
	D. L. Murphy	Chief, Clinical Neuropharmacology	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoamine oxidase (MAO) activity and the average evoked response measure of augmenting/reducing (AR) differentiate some psychiatric patient groups from normal controls. This study tested whether a combination of MAO and AR predict psychiatric vulnerability in a nonhospitalized and previously undiagnosed sample. Almost 400 college males were screened on the Minnesota Multiphasic Personality Inventory (MMPI). A subsample of students with indications of psychological problems was selected as was a control sample. These students were classified into 4 biologically homogeneous groups--low MAO/augmenting, low MAO/reducing; high MAO/augmenting, high MAO/reducing. Based on the Research Diagnostic Criteria (RDC), more psychopathology is found among low MAO/augmenting and high MAO/reducing students. This result replicates the pattern indicated in a previous study and suggests that specific biological variables may be related to a general vulnerability to psychiatric problems. This possibility is supported by the results of an 18 month follow-up which shows that low MAO/augmenting and high MAO/reducing students continue to report new episodes of hypomania and major depression.

PHS-6040
(Rev. 10-76)

Project Description:

This project was designed to identify people who have experienced psychological difficulties or psychiatric symptoms but who have never been hospitalized, treated, or diagnosed and then to test whether there are biological parallels between such cases and psychiatric inpatients.

To generate the sample, 385 college males were screened on the Minnesota Multiphasic Personality Inventory (MMPI). Everyone with aberrant scores and a control sample with normal scores also completed monoamine oxidase (MAO) testing, average evoked response (ER) testing, and structured interview with a psychiatrist using the Research Diagnostic Criteria (RDC).

Eighty-two percent of the cases with aberrant MMPI scores met the RDC for at least one diagnosis, whereas only 22% of the controls met any RDC. All subjects were classified into 4 biologically homogeneous groups--low MAO/augmenting, low MAO/reducing, high MAO/augmenting, and high MAO/reducing. The frequency of RDC diagnoses in each biological group showed that most psychopathology was found among low MAO/augmenters and high MAO/reducers. A similar pattern was noted among psychiatric patients.

We conclude that a research sample identified by MMPI and RDC screening of a nonhospitalized group yields many individuals with some psychopathology. That these individuals show a specific biological parallel to psychiatric patients and may be vulnerable to future psychiatric problems.

A follow-up of the student subjects has further indicated that the biological groups predict future psychiatric problems, particularly hypomania and major depression episodes.

This project is significant to the Institute's research program because it may have implications for diagnosing psychiatric disorders using biological variables and for population screening studies of mental health.

Publications:

Haier, R.J., Buchsbaum, M.S., and Murphy, D.L.: Screening young adults for psychiatric vulnerability: A preliminary comparison of biological and clinical measures. Psychopharm. Bull. 15(1): 7-9, 1979.

Haier, R.J., Rieder, R.O., Khouri, P.J., and Buchsbaum, M.S.: Extreme MMPI scores and the Research Diagnostic Criteria: Screening college males for psychopathology. Arch. gen. Psychiatry 36: 528-534, 1979.

Haier, R.J., Buchsbaum, M.S., Murphy, D.L., Gottesman, I.I., and Coursey, R.D.: Psychiatric vulnerability, monoamine oxidase, and the average evoked potential. Arch. gen. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00493-03 LPP

PERIOD COVERED October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of thought disturbance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP NIMH
OTHER:	H. Weingartner	Acting Chief	LPP NIMH

COOPERATING UNITS (if any)

Ward 4E, BPB, NIMH

LAB/BRANCH Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated during this report period.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00494-03 LPP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical and Empirical Detection of Simulated Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D.Rosenthal	Research Psychologist	LPP, NIMH
	R. Haier	Research Psychologist	LPP, NIMH
	Paul Alexander	Assistant Professor of Psychiatry	Brown Univ.
	Victor Reus	Clinical Associate	BPB, NIMH

COOPERATING UNITS (if any)

St. Elizabeth's Hospital, Psychodrama Section

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project explores the ability of psychiatrists and others in differentiating actors simulating schizophrenia from schizophrenic patients, using a 30-minute interview. The initial stage of the project has been completed. Twenty-seven subjects (15 "actors" and 12 patients) were interviewed by an NIMH psychiatrist, and he identified 24 of the 27 subjects correctly. Videotapes were made of the interviews and segments of the tapes have been shown to audiences composed of psychiatrists and other raters. The ratings, based on the videotapes, were not judged to be adequate for analysis and the project was terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00495-03 LPP

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The psychobiology of cognitive processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Laboratory of Clinical Science,DCBR,NIMH
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LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been designed to explore the psychobiology of cognition in man. This research attempts to interrelate psychological and biological determinants of various components of cognition such as the specific and discrete psychobiological mechanisms that define the storage, encoding or elaboration, memory, and retrieval of experience. Experiments are designed to examine the changes in cognitive processes in response to pharmacological and behavorial manipulations. Some of these studies have examined whether drugs that enhance aspects of cognition would reverse cognitive disturbances in patients. Findings include the cognitive changes (encoding, memory, retrieval) that occur in response to drug treatments that alter the activity of cholinergic and noradrenic central nervous system activity in patients with various psychiatric disorders and normal controls. Cognitive changes produced by neuropeptides in depressed patients and controls have also been evaluated. The patient groups studies included those with disturbances in mood, various forms of dementias, including Huntington's disease, Korsakoff's psychoses and Alzheimer's disorder, hyperactive children, and schizophrenic patients.

Project Description:

The research projects reviewed here are all concerned with the psychobiology of cognitive processes in man. Specifically, the projects have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies have examined the psychobiological processes that define the acquisition and storage of new experience, how processed events are altered, or elaborated in memory, and the mechanisms that are involved in retrieval of stored information. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information in long-term memory, the kinds of state or trait specific cognitive strategies used in the elaboration and transformation of information, organization in memory, and the kinds of strategies that subjects use to retrieve experience that they have stored in memory.

These research efforts have utilized two broadly conceptualized strategies for defining the psychobiology of cognitive processes. One is to contrast the effects of different treatment strategies on various components of cognition, such as on information storage, consolidation, encoding and retrieval. These studies include attempts to systematically alter cognition through, 1) pharmacological manipulations, such as cholinergic drugs, noradrenergic drugs, neuropeptides, and, 2) by behavioral manipulations that alter, arousal/activation, stimulus attributes (altering encodability) and types of stimulus processing strategies subjects use to process information. These studies are carried out in normal man, as well as in patient groups with different forms of psychopathology. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting manipulations of different brain systems producing different cognitive effects would help provide a picture of structure of the psychobiology of cognition. The alternative strategy for researching the psychobiology of cognition is to contrast the different forms of cognitive failures seen in different psychiatric disorders. Would the disrupted cognition seen in some psychopathological states be qualitative and quantitatively different and related to specific changes in central nervous system activity? For example, how are the amnesic/cognitive disorders seen in Huntington's disease patients, Korsakoff syndrome, Alzheimer's disorder, different, and how might differences be an expression of the specificity of central nervous system involvement in each of these disorders? How are the mechanisms that account for cognitive disruptions different or similar in these different disorders? Some studies that are part of this project have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments as these might attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity syndrome in children, depression, mania and the schizophrenias. Each of these studies that make up this research project can be seen as clinically relevant, as well as pertinent for understanding unimpaired cognitive processes.

In summary, all of the research studies that are part of this project are involved in defining what are the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, and retrieval of experience, and include both basic studies and clinical investigations.

1. Behaviorially defined mechanisms that alter components of cognition.

Characteristics of stimuli such as, a) imagery, b) emotional arousing properties, c) information presentation rate, d) mode of processing, 3) language vs. pattern information, have been studied in relation to its effects on acquisition (learning), strength of learning, and components of information retrieval. The studies have examined these factors in normal controls, as well as in patient groups (depressed patients, hyperactive children, patients suffering from various forms of dementia). Some of the issues raised in these studies include the following. How might aspects of information processing alter the attentional, short-term memory, encodability and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures to learn and remember do so because of disruptions in some, but not all, of these component cognitive processes, and can manipulations of some characteristics of information processing change these disruptions in cognition?

2. Pharmacological alterations (enhancement and disruption) of cognitive processes.

These studies have contrasted the cognitive enhancing effects of cholinergic agents such as arecoline, physostigmine, lecithin treatment with those of amphetamine in both normal subjects, as well as in patients (hyperactive children, Alzheimer's disorder, depressed patients). These studies have also included investigations of cognitive response to neuropeptides. Would different kinds of psychoactive drug treatments that produce qualitatively different central nervous system responses produce systematically different changes in cognitive processing? That is, would enhancements or disruptions of cognition occur through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernable? This research has also involved attempts to find clinically useful strategies for altering disrupted cognition in man.

3. State-dependent learning. This area of research provides a useful framework for exploring:

   a) The qualitatively unique manner in which events are stored (encoded) and retrieved from memory.

   b) Studies of disturbances in mood state and how these define mood specific strategies for processing experience and remembering past events in memory.

   c) Qualitative changes in cognition in response to psychoactive drugs.

   d) Contextual factors as determinants for defining the nature of trace events in memory.

4. Cognition and mood. Studies have included research of mood-related changes in: 1) the brain lateralization of cognitive functions; 2) arousal and activation and its role in information processing; and 3) the encoding and retrieval of events in normal mood and while depressed or manic. This research has examined how patients with disturbances in mood, process information in a mood state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes.

5. Memory Consolidation. This research has focused on the psychobiological events that follow the acquisition (storage) of information and well before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory, and the processes that might further sustain, enhance or disrupt stored trace events that are already part of memory. The rate of decay of information, the susceptibility of information to interference, and biological factors may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may be important in understanding cognitive failures in the dementias.

6. Cognitive disruptions in the dementias. Studies have examined and contrasted the specific mechanisms and structures that define cognitive failures in different neuropsychiatric populations. Studies of components of cognition that are affected in Huntington's Disease have been systematically explored and contrasted to cognitive processing in Korsakoff psychosis, Alzheimer's disorder, and other neuropsychiatric disorders. These studies have attempted to discover whether much of the cognitive failures seen in these syndromes may be due to encoding (acquisition failures), unreliable access to information which is in memory, interference factors, and disruptions in consolidation.

Other studies have involved systematic treatments with various psychoactive drugs that may prove useful in reversing aspects of the cognitive changes that define a particular dementia, and uncovering what underlying psychobiological mechanisms may be disrupted in each of these syndromes.

Methods

A large variety of cognitive strategies have been designed to explore the psychobiological components of cognition in the studies that are part of this project. These strategies have included modified forms of current methods used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of:

a) organization of information (sorting of information, labeling or coding clusters of events such as words, pattern information varying in structure);

b) alterations in the informational context for processing information;

c) stimulus attributes (imagery, emotional properties, frequency, mode of presentation);

d) various forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning);

e) processing time and type of presentation of information;

f) type of processing strategy (processing on the basis of meaning or sound properties);

g) immediate vs. delayed recall of information with or without rehearsal of stored information;

h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization);

i) rapid (tachistoscopic) presentation of information;

j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings."

k) measures of very long-term retrieval.

Major Findings

Studies of psychobiological determinants of cognition in unimpaired subjects.

In a number of experiments we have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and its retrieval. Arecoline, physostigmine, and lecithin treatments produce enhancements in the storage and retrieval of information. Scopolamine treatments which disrupt cholinergic activity, produce an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated patients with Huntington's Disease.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in an undrugged state, and afterwards are treated with arecoline, there is a comparable facilitation of information later recalled, when compared to learning and recall that occurs under arecoline treatment conditions.

While cholinergic treatments alter how much information is stored and later retrieved from memory, these effects are also state dependent. That is, there appear to be qualitative cholinergic drug state specific differences in how experiences are encoded and how retrieval of past experience is accomplished.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input process under drug state conditions. Unlike cholinergic manipulations amphetamine eappears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients; hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent learning and state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine and the cognitive disrupting effects produced by scopolamine vs. lithium has served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

Some behavioral strategies have also been used to investigate the specificity of cognitive processes as these might be involved either separately or together in the acquisition, elaboration and amplification, trace maintenance in memory and retrieval. The emotional properties of stimuli seem to amplify or strengthen what is learned or stored in memory in a manner similar to that produced by drug treatments such as amphetamine. Reinforcement following information processing also acts to strengthen events already in memory storage. Other behavioral determinants, such as word imagery, presentation rate, familiarity, stimulus structure, appear to alter how much is learned, rather than the strength of learning, in a manner similar to the effects of increased cholinergic activity.

Clinical studies of the psychobiological determinants of cognitive processes.

Clinical studies have been designed to investigate the specificity of changes in components of cognitive processes in adults with disturbances in mood, schizophrenic patients, hyperactive and learning-disabled children, and patients with different forms of dementia. The studies that have contrasted the different forms of disrupted cognition in Huntington's Disease (HD), Korsakoff's psychosis, Alzheimer's disorder, and other neurological disorders

1) HD patients fail to consistently remember information which is, nevertheless, in memory store.

2) HD patients demonstrate retrieval failures which are due to inadequate encoding of information during acquisition. They fail to use slow presentation rate, semantic encoding, imagery and other attributes of stimuli to more effectively and elaborately encode information. Some drug and behavioral treatments may prove helpful to normalize this encoding disruption.

3) Alzheimer's patients also fail to "appreciate," learn and retrieve experiences. These patients have access to appropriate semantic structures in memory but fail to use these in organizing experiences so that they might prove memorable.

4) Alzheimer's patients appear to show a small but measureable cognitive response (improvement) following cholinergic and amphetamine treatment. Recall of organized information is most facilitated while rote-like learning seems unaltered. It is as yet uncertain whether the cholinergic response is qualitatively different from that seen following amphetamine treatment. These patients who have also been treated chronically with a cholinergic drug, have appeared to show a temporary improvement in cognitive functions, but this enhancement is not a sustained drug response. Strategies for defining cognitive changes in Alzheimer's disorder, and potential treatment strategies are relevant issues in the study of normal aging processes.

5) Recent studies testing the cognitive changes that follow treatment with vasopressin-like compounds (DDAVP) may provide us with some new strategies in the treatment of dementias. We have shown that DDAVP produces a marked and sustained cognitive enhancement in depressed patients, as well as in normal controls. The form of that cognitive enhancement appears to act through altering organizational mechanisms which may be particularly relevant for the kinds of disruptions in cognition seen in Alzheimer's patients. Studies of the structure of cognitive changes in response to neuropeptide treatment should prove helpful in defining biological mechanisms that are important for those cognitive processes involved in the organization and elaboration of information in memory.

6) Recent completed cognitive studies of depression have shown that these patients demonstrate learning memory failures when they are required to organize information, but show no such deficit when organization is present. That is, when information is presented in an organized fashion, then depression-related cognitive deficits are not apparent. In some respects, their cognitive disruption is similar to that seen in Alzheimer's disorder. These patients also demonstrate qualitative changes in how they encode events and the strategies by which they recollect previously learned experiences (state-dependent learning). Some of these effects may be related to alterations in lateralization of cognitive processes.

7) Studies of cognitive processes in children have demonstrated some of the specificity of cognitive disturbances that are apparent in hyperactive and learning-disabled children. Hyperactive children appear to learn (store) about the same amount of information as normal controls (under experimental conditions). However, unlike controls, retrieval of that information is highly dependent on the presence of strong environmental cues or prompts at the time remembering takes place. Both normal and hyperactive children respond with enhanced cognition following amphetamine treatment. However, those aspects of cognition that are enhanced in these different children are qualitatively different. The facilitation of cognitive functions in the hyperactive child is one which would not readily translate into more effective school performance, since amphetamine increases learning and recall of superficial characteristics of information processing. This is in contrast to an amphetamine-induced increase in learning and recall of meaningfully processed information in the normal child. It, therefore, appears useful to integrate behavioral retraining strategies along with stimulant treatment to effectively help the hyperactive child.

8) Recent studies examining learning and recall in stimulant-treated hyperactive children have also shown that learning and retrieval of learning is stimulant drug state specific (state-dependent learning). Although learning may be enhanced following amphetamine treatment, its effective retrieval is largely dependent on reinstitution of the drug state present at the time of learning. This has clear implications for the long-term effects of stimulant treatment in hyperactive children, particularly in how this effects their school performance.

An integrative system for exploring the psychobiology of cognitive processes.

Ongoing studies have yielded findings which permit us to examine how: a) specific aspects of cognition are altered by drugs that effect activity in different parts of the central nervous systems, and b) different forms of neuropathology and psychopathology lead to different alterations in the cognitive processes that normally would lead to successful appreciation, acquisition and retrieval of experience. The extensive findings from the studies described here have suggested a pattern to the pharmacological, behavioral, and clinical finding that suggests that distinctive psychobiological mechanisms are involved in the organization encoding, consistent retrieval, and consolidation of information in memory. Different behavioral-neurochemical determinants may alter the strength of trace events in memory, their accessability, and the amount of stored experience in memory.

Dissemination of Information:

I. Psychonomic Society - 19th Annual Meeting, San Antonio, Texas, November 9-11, 1978 - "Neuropsychopharmacological studies of human learning and memory."

II. NINCDS Huntington's Disease Symposium, San Diego, California, November 16-18, 1978 - "Encoding-Retrieval Processes in Huntington's Disease."

III. American College of Neuropharmacology, Maui, Hawaii, December 13, 1978 - "Stimulant Drug Responses of Normal and Hyperactive Children and Adults" and Panel on "Psychobiological Issuesin Children."

IV. American Psychiatric Association - 132nd Annual Meeting, Chicago, Illinois, May 12-18, 1979 - "Drug-induced enhancement of human learning."

V. American Psychological Association - 87th Annual Convention, New York, N. Y., September 1-5, 1979 -

   1. "Memory Consolidation: Psychopharmacological, Physiological and cognitive Aspects"

   2. "Encoding Processes in Depression"

Publications:

Weingartner, H., Murphy, D. L., and Stillman, R. C.: Drug and mood state specific encoding and retrieval of experience. In International Challenge of Drug Abuse. NIDA Monograph 19, DHEW/Pub. ADM-78-654, 1978.

Weingartner, H., Caine, E. D., Ebert, M. H.: Imagery, encoding and the retrieval of information from memory: Some specific encoding-retrieval changes in Huntington's Disease. Journal of Abnormal Psychology, Vol. 88 No. 1, 52-58, 1979.

Weingartner, H., Murphy, D. L., and Stillman, R. C.: Mood State-Dependent Learning. Stimulus Properities of Drugs: Ten Years of Progress, Janssen Research Foundation, 1978, Elsevier North-Holland, Inc., New York, 445-453, 1979.

Reus, V. I. Targum, S. D., Weingartner, H., and Post, R. M.: Effect of lithium carbonate on memory processes of bipolar affectively ill patients. Psychopharmacology (in press).

Sitaram, N., Weingartner, H., and Gillin, J. C.,: Human serial learning: enhancement with arecoline and choline and impairment with scopolamine correlate with performance on placebo. Science, 201: 274-276, 1978.

Eich, J. E., Weingartner, H., Stillman, R. C., and Wyatt, R.: State-dependent retrieval of item associative and serial-order information. First International Symposium on Drugs as Discriminative Stimuli Journal (in press).

Reus, V. I., Silberman, E., Post, R. M., and Weingartner, H.: D-Amphetamine: effects on memory in a depressed population. Biological Psychiatry, Vol. 14, No. 2, 345-356, 1979.

Weingartner, H., Sitaram, N., and Gillin, J. C.: The role of the cholinergic nervous system in memory consolidation, Bulletin of the Psychonomic Society Vol. 13, (1), 9-11, 1979.

Weingartner, H., Caine, E. D., and Ebert, M. E.: Encoding processes in Huntington's Disease. Raven Press (in press).

Sitaram, H., Weingartner, H., and Gillin, J. D.: Choline Chloride and Arecoline: Effects on Memory and Sleep in Man. Edited by A. Barbeau, J. H. Browdon, and R. J. Wurtman. Nutrition and the Brain, New York, Raven Press, Vol. 5, 267-375, 1979.

Altered Cognitive Processes in Children: Psychobiological Approaches, in B. Shopsin and L. Greenhill (Eds.). The Psychobiology of Childhood: Profile of Current Issues, Spectrum Publications, Inc., (in press).

H. Weingartner, Murphy, D. and Martello, J.: Mood State-Dependent Learning following the experience of success or failure, American Journal of Psychology (in press).

Weingartner, H., Rapoport, J., Buchsbaum M, Ebert, M. H., Mikkelsen, E. and Caine, E. D.: Cognitive processes in normal and hyperactive children and their response to amphetamine treatment. Journal of Abnormal Psychology (in press).

Reus, V. I., Weingartner, H. and Post, R. M.: Clinical implications of state-dependent learning. American Journal of Psychiatry, Vol. 136:7, 927-931, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00496-03 LPP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Animal Models of Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.M. Anderson	Staff Fellow	LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of my research is to gain a better understanding of human psychopathology through the study of biochemical and behavioral variation in lower animals. Presently this objective is being pursued through a study of normal variation in biogenic amine metabolism. Monoamine oxidase (MAO) activity has been examined in brain and liver of selected inbred strains of mice. Variability in MAO activity was demonstrated among the strains, but the nature of the inheritance of these differences has not been studied. The rank ordering of the strain means suggests a high correspondence between liver and brain MAO activity as well as a high positive association between the activities of the A and B forms of MAO, as measured with the substrates phenylethylamine, serotonin and tyramine. Environmental variables and genotype-environment interactions have also been found to be of importance in controlling activity levels of MAO.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00499-02 LPP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biological and Family Studies of Chronic Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Ronald O. Rieder	Research Psychiatrist	LPP, NIMH
Other: E. Donnelly	Research Psychologist	St. Elizabeth's Hospital

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to determine if certain biological factors, namely abnormalities on the CAT Scan, low platelet MAO, or left-handedness have an etiological relationship with schizophrenia. The method involves finding schizophrenics with one of these factors and studying the relatives to see if the factor and schizophrenia segregate together in these pedigrees.

After the collection of the CAT Scan data on the sample of 20 schizophrenics, the work on this project shifted to study the meaning of those abnormalities found. Neuropsychological testing, including the Halstead-Reitan Battery, was administered to those schizophrenics with and without CAT Scans showing enlargement of the sulci (cortical atrophy) and ventricular enlargement. A significant relationship appears to exist between performance on the Halstead-Reitan Battery and enlargement of the sulci among these young chronic schizophrenics. Sulcal enlargement was not commonly found (4 cases of 20), but may indicate a previously unrecognized organic brain impairment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00671-10 LSES

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Social Origins of Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard I. Pearlin	Research Sociologist	LSES NIMH
Other:	Clarice Radabaugh	Social Science Technician	LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the relationship between social experience and psychological well-being. It looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two times. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed, and a sub-sample (1200) of this group was reinterviewed in 1976-77. Analyses have been made to identify the events that are particularly likely to result in emotional distress, and to understand how people use psychological and social resources to cope with the problems and vicissitudes of life.

PHS-6040
(Rev. 10-76)

Project Description:

This is a longitudinal study of social stress spanning a period of about four years. The first wave of interviews was conducted in 1972 with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of people's lives: 1)the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; 2)the coping strategies people employ in dealing with life-problems; and 3)symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions that initially asked about chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change on these dimensions of people's lives. Newly constructed questions sought information about key life-events that occurred since 1972. One type of event is represented in the many changes and role transitions that are normative aspects of the life-cycle. A second type of event is represented in the more unexpected, non-normative disruptions of important roles and activities, such as divorce, serious illness, involuntary unemployment, and other life crises.

Together the two interviews provide a large body of information about problems woven into the fabric of major social roles, the gains and losses involved in life-cycle transitions, the crises that intrude into people's lives, coping repertoires and psychological outcomes. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to learn how people attempt to avoid being psychologically harmed by such experience.

During the past year our work proceeded along both methodological and substantive lines. The major methodological task has been the development and refinement of all of the measures for which we have longitudinal data. The presence of error in such measures can seriously distort the assessment of change over time. Consequently, it is of vital importance in longitudinal studies to identify and control error. This involves the use of an elaborate series of statistical steps that have been embodied in a complex computer program. We have completed this phase of our work, enabling us to evaluate life-changes with a high level of reliability.

Our substantive efforts were primarily directed at further explorations of the conditions that people experience at different phases of the life-course. There is an established view that family transitions, in particular, represent important turning points in the lives of people. These transitions include such key events as being married, having children, seeing children move through various stages of development, the launching of children from the household, becoming a grandparent, and being widowed. It is frequently assumed that the major reorganization of life demanded by events and transitions of this sort may result in severe emotional distress.

Consequently, until readjustments are worked through, family transitions seem to be a likely source of psychological disturbance. Contrary to these expectations, however, family transitions--with the exception of being widowed--have virtually no effect on psychological functioning. One apparent reason for their lack of impact concerns their predictability. By being so closely linked to the life-cycle, transitional events are typically forecast in advance of their occurence. Much of the adjustment to these events, therefore, may take place anticipatorily, thus minimizing the effects of the actual event.

In contrast to family transitions, certain life-changes that are less easily forecast do exert an emotionally disruptive effect. Outstanding in this respect are the following: involuntary loss of a job, especially for reasons of poor health; divorce and separation; the death or serious illness of a child; and, as earlier noted, being widowed. When allowances are made for prior emotional states and when effects of major social characteristics are controlled, these are the events that stand out as being most potent. These particularly powerful events notwithstanding, people are generally found to adopt change with considerably more ease than might be expected from theories of stress that emphasize the importance of equilibrium to psychological well-being.

We have continued to test certain assumptions and practices found in studies of the aging process. One such assumption, more implicit than explicit, is that aging is inherently difficult. Both the accumulation of unwanted changes through time and the inevitable approach toward death would seem to make it an intrinsically stressful process. However, it is not; in fact, stress generally declines with age, at least up until the time that people become infirm. The challenges and problems of assuming new roles make the earlier years of adulthood more stressful than the later years.

Findings such as these point up the methodological advantage of making comparisons across different age ranks rather than following the more common strategy of separately studying single age groups. Comparative perspectives are essential to discovering the circumstances that are distinctive of a particular life-stage and those that are not. In addition, we have learned that it is imperative to make comparisons within as well as across age groups. It is a repeated finding that being of the same age does not override the social and economic differences among people. That is, people belonging to a cohort do not necessarily experience similar conditions of life because they are the same age. Instead, their experiences depend on their social and economic characteristics. It is indefensible, therefore, to speak of age groups as though they have traversed a uniform life-course.

In the next year we shall examine certain life events through the use of formal causal analytic techniques. We shall be particularly concerned with divorce and separation, attempting to identify some of its causes, the mechanisms through which it influences the lives of people, and its emotional consequences. A second objective of our work will center on coping.

Previous analyses have dealt exclusively with modes of coping with relatively enduring life problems. By contrast, future work will be primarily concerned with the ways people cope with change.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00672-14 LSES

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Social Psychological Correlates of Occupational Position

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. L. Kohn	Chief, Lab of Socio-environmental Studies	LSES	NIMH
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11.45	5.65	5.80

CHECK APPROPRIATE BOX(ES)

☒(a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the re-interviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. The study has also been replicated in Poland.

Project Description:

The principal goal of this research is to assess the reciprocal relationships between occupational conditions and psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study is to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of wives is designed to ascertain whether occupational conditions affect men and women similarly. (On this, see last year's report.) These data should also enable the investigators to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children is designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts have been focused on eight objectives: (1) Using the longitudinal data for men, analyzing the reciprocal relationship between job conditions and psychological functioning. (2) Using the data from both the men and their wives, beginning an analysis of the relationship between occupational conditions and the use of leisure time. (3) Assessing gender differences in the occupational determinants of job satisfaction. (4) Beginning an analysis of the relationship between housework and psychological functioning. (5) Examining the relationships between the structural imperatives of jobs and their rewards. (6) Beginning the analysis of a thoroughgoing replication, in Poland, of the U.S. findings on the interrelationship of social class, occupation, and psychological functioning. (7) The initiation of another cross-national replication in Japan. and (8) Reassessing the relationship, in the U.S. and in several other countries, between social class and parental valuation of self-direction or conformity to external authority.

1. Longitudinal analysis of the reciprocal relationship between job conditions and psychological functioning. The annual report of two years ago described Melvin Kohn and Carmi Schooler's analysis of the reciprocal effects of one pivotal facet of work (its substantive complexity) and one pivotal facet of psychological functioning (intellectual flexibility). Last year's report described the extension of this causal model, to encompass not only the substantive complexity of work, but also the entire set of job conditions that the investigators had earlier found to be pertinent to psychological functioning. (They have termed this set of occupational conditions "the structural imperatives of the job.") In further analysis this year, Kohn and Schooler refined the measurement models for several other facets of psychological functioning -- in particular, authoritarian conservatism, anxiety, trust, self-confidence, self-deprecation, idea-conformity, fatalism, and standards of morality. They then developed causal models to assess the reciprocal effects of the structural imperatives of the job and each of these facets of psychological functioning. These models show that job conditions both affect and are affected by all facets of psychological functioning that have been studied. In general, the conditions determinative of occupational self-direction -- namely, the substantive complexity of work, routinization, and closeness of supervision -- have the most far-reaching effects; but position in the organizational structure, job pressures, and extrinsic risks and rewards matter also for psychological functioning.) Now in process is the development of a more encompassing model, which includes not only the entire set of job conditions but also several facets of personality, considered simultaneously. So complex a model is exceedingly difficult to estimate, but substantial progress was made this year. Next year's report will presumably have more to say about this analysis.

2. Occupational conditions and the use of leisure time. The purpose of this analysis is to examine the relationship between job conditions and how workers use their discretionary time -- time not committed to earning a paycheck or to other activities necessary for meeting the basic physical needs of the family. Other analyses have found that job conditions both affect and are affected by subjective psychological processes, but this is the first research to focus in depth on off-the-job behavior. The hypothesis is that job experiences affect and are affected by what people actually do with their time off the job, just as job experiences affect and are affected by what people feel, believe, and value.

One of the main purposes of this inquiry -- being conducted by Karen Miller and Melvin Kohn -- is to investigate the mechanism of transfer between these two spheres of life, work and leisure. A major issue in the field, to which this research is addressed, is whether workers generalize from or compensate for the rewards and deprivations of work by their behavior off the job. It is of course also possible that the processes are more complicated than is implied by either a direct generalization or a compensation model.

Miller and Kohn conceive of leisure-time activities as having a number of dimensions, just as do work conditions and psychological processes. One pivotal dimension is intellectual demandingness, that is, the extent to which people's leisure-time activities place demands on their intellectual resources. Other major dimensions of leisure-time activity are how physically sedentary or active, how solitary or social, how varied or non-varied, and how competitive or non-competitive it is.

Miller and Kohn plan to test the generalization vs. compensatory hypotheses by relating occupational conditions to these several dimensions of leisure time. For example, if people directly generalize between job and non-job activities, substantively complex work should be positively related to intellectually demanding leisure time, but if people compensate, then substantively complex work should be inversely related to intellectually demanding leisure-time pursuits. The investigators will also ask: do people who do physically heavy work compensate by being sedentary off the job, or do they generalize by choosing physically active pursuits? Does routine work relate to varied or non-varied leisure-time activity? Does teamwork on the job relate to social or solitary leisure-time activities? Do work situations requiring competition relate to competitive or to non-competitive activities outside the work setting?

As a necessary first step in answering these questions, the investigators are building measurement models of the major dimensions of leisure-time activity. They have focused first on the intellectual demandingness of leisure time, developing measurement models of this concept for both men and women. The indicators are hours spent watching television, frequency of attendance at plays, concerts, and museums, number of books read, time spent on hobbies, intellectual level of magazines read, and intellectual level of newspaper reading. The model for men incorporates both 1964 and 1974 data, while the model for women is necessarily limited to 1974.

For the men, hours spent watching television makes a negative but weak contribution at both times, while intellectual level of magazines and newspaper reading are the dominant indicators. Attendance at plays, number of books read, and hobbies are intermediate in strength. The ten-year overtime correlation of the intellectual demandingness of men's leisure-time pursuits is an astonishingly high 0.96.

For employed women, the measurement model is similar to the men's in some respects, different in others. Television again makes a weak negative contribution; in this case, it is statistically nonsignificant. However, the variables that were intermediate in strength for men -- plays, books, and hobbies -- are dominant for employed women. The dominant indicators for men -- magazines and newspapers -- are of lesser importance for employed women. Miller and Kohn believe that the explanation for a sex difference in the measurement model for this aspect of leisure-time, but not for any psychological concept measured thus far in the research, lies in the indicators being time-linked. (It is well established that employed women have exceptionally limited time available for leisure.) The most plausible hypothesis, borne out by the correlations of the indicators with the number of hours worked in the average week, is that variation in hours worked for women and variation in their household responsibilities for children, etc., explain the higher intercorrelation among time-consuming activities for employed women than for employed men.

The measurement models for the intellectual demandingness of leisure time, together with the pertinent occupational conditions and background variables, provided the basis for causal models assessing the reciprocal relationships between occupational conditions and intellectual leisure-time activity. The assessment of effects can be made with more confidence for the men than for the women, because the men's data are longitudinal. For the men, almost all effects are contemporaneous rather than lagged. The effect of the substantive complexity of work on the intellectual demandingness of leisure-time activities is by far the strongest. Job protections and income have small positive effects, and hours worked has a small negative effect, on leisure-time use. In the reverse causal direction, intellectual leisure-time use affects only substantive complexity. Thus, those who engage in intellectually demanding leisure time activities mold their jobs to be more substantively complex. This may appear to be inconsistent with a previous finding that intellectual flexibility, as assessed in the interview situation, has a lagged but not a contemporaneous effect on the substantive complexity of work. The reason for this difference may be that intellectual functioning in the interview situation measures a potential for intellectual activity, whereas the intellectual demandingness of leisure time measures a style of activity. People who seek out ways to be intellectually active in their off-the-job activities may also seek out ways to be intellectually active on the job.

Miller and Kohn's main conclusion for the men is that substantive complexity is the strongest determinant of the intellectual demandingness of their leisure time. The causal analysis for the women is in a preliminary stage, but it appears that substantive complexity may be an important determinant of the intellectual demandingness of women's leisure-time use, too. These results provide support for the direct generalization hypothesis over the compensation hypothesis. It remains to be seen whether this same learning model holds for other dimensions of off-the-job behavior, to be explored in the next phase of the analysis.

3. Gender differences in the occupational determinants of job satisfaction. Because the subjective evaluation of job experiences involves both the cognitive appraisal of job conditions and a hierarchy of values that determine awareness and standards, individual attributes are often used to explain variation in job satisfaction. If there are basic differences in what is sought from employment, then satisfaction with work can be viewed as conditional on these individual definitions or needs. Joanne Miller's analysis focuses on gender differences in job satisfaction. Men's and women's orientation to work is said to differ in fundamental respects. Differential socialization before entry into the labor force, dissimilar parental and conjugal roles, and systematic differences in occupational opportunities suggest that the meaning ascribed to work and rewards sought may vary by sex. The same job conditions may be more or less central for job satisfaction because male and female workers differ in their orientation to work.

Job satisfaction is defined as the cumulative subjective evaluation of work experiences. As such, the content of job satisfaction can vary by the salience of particular features of the job and also by the importance attached to these dimensions. Nineteen specific criteria for judging work were included in Kohn and Schooler's occupational survey. In an effort to capture both the content and intensity of judgements made about work, respondents were asked about their degree of satisfaction (or dissatisfaction) with the specific aspects of their jobs they deemed important. The index is an average of these judgments, weighting a person's satisfaction with an aspect of work he deems very important twice as much as his satisfaction with an aspect of work he considers only fairly important. This approach assumes that a job is not evaluated as a single entity but as multi-dimensional tasks performed in a particular physical and social context.

This operationalization of job satisfaction immediately focuses the analysis on possible gender differences in the content of occupational satisfaction -- which aspects of work are most important to men and which to women? Miller finds that both men and women consider a broad range of job characteristics important. Overall, men and women seem to judge work along similar dimensions -- especially in terms of economic advantage, interest, pride, and relationships with other people in the immediate work setting. The sex of a worker is not systematically related to the importance placed on pay, fringe benefits, how interesting the work is, the chance for exciting work, the supervisor, co-workers, opportunity to feel pride in craftsmanship, or job security.

The major difference in work values concerns positional authority. Men are more likely to emphasize the opportunity to exercise leadership, make decisions, and get ahead, while women are more likely to find the chance to help people an important non-financial reward. Another gender difference in judging work involves the convenience aspects of the job. Hours, cleanliness, how tiring the work is, and job pressure are of more concern to women than to men. But this difference in emphasis is not entirely clearcut. Although men are less likely than women to focus on hours worked (in fact, there is relatively little variation in the length of the work week for men) they are more likely to feel that "the amount of freedom" they have is an important criterion. If "freedom" includes flexibility in scheduling or ordering tasks and in discretionary time of arrival, men may also be expressing, in part, some concern with convenience aspects of work.

To the extent that women's criteria for judging work are systematically different from men's, one might expect gender differences in the determinants of job satisfaction. The composite job satisfaction index used in this analysis reflects individual work values as well as cognitive assessments by weighing work evaluations by the degree of importance the respondent attaches to the job characteristic. However, even if men and women's orientations to work were identical, adjustment to job conditions could still be contingent on gender. In this part of the analysis, Miller examined how various aspects of work are related to job satisfaction. She focused on those job conditions that are most central to the nature of the work performed. These include job conditions that determine the level of occupational self-direction, job pressures, organizational structure and position, job uncertainties, and rewards and protections of the work. The analysis shows a strong relationship between day-to-day job experiences and job satisfaction, regardless of the worker's gender. However, socially selective recruitment into and retention in particular occupations could explain the relationships observed between job conditions and job satisfaction. To the extent that job satisfaction is predetermined by educational experiences, job conditions, per se, could have different net effects. Other background characteristics may also be related to occupational selection, particular employers recruiting more heavily from urban or from rural populations, from particular racial, religious, national, or socio-economic backgrounds, or from older or younger members of the labor force. It is also possible that there is selection into jobs on the basis of a psychological predisposition to be satisfied or dissatisfied.

Since selection processes are of particular concern in this analysis, the detailed comparison of the effects of current job conditions on the satisfaction of male and female workers statistically controls earlier job satisfaction and background characteristics. Even under these stringent controls, there is a strong relationship between job conditions and worker satisfaction for both men and women. Miller showed that intrinsic aspects of work are stronger correlates of job satisfaction than either the extrinsic rewards of the job (income and job protections), job uncertainties, or the background of respondents, regardless of sex. But, although both men and women evaluate their jobs in terms of the work performed, there are differences in the particular job conditions that influence their assessments. Women emphasize the substantive complexity of work, dirtiness, and number of hours worked, while men focus on closeness of supervision and position in the organizational structure. Time pressure is important for both men and women. Miller also demonstrates, in reciprocal causal models, that the significant effects of job conditions on job satisfaction cannot be attributed to job molding -- i.e., to satisfied employees carrying out their work differently from dissatisfied workers.

These findings confirm that day-to-day job conditions determine worker satisfaction and that occupational self-direction is an important criterion for judging work, regardless of sex. There is some evidence that individual work values can alter the subjective nature of work experiences. For example, job pressures are more central for women than for men and positional authority is more important for men's job satisfaction. However, the broad range of job conditions that affect job satisfaction is equally impressive for men and women.

4. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of work conditions encountered in paid employment outside the house, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of housewives, but also of working wives and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have developed measurement models of the basic conditions of work encountered in housework. Using confirmatory factor analysis, they have developed models of: time spent on housework, the substantive complexity of household work, closeness of supervision (by one's spouse), and routinization. The relationships between concepts and specific indicators are different for men and women; in general, these differences parallel the traditional sexual division in housework responsibilities. Thus, the substantive complexity of the work men do around the house is strongly characterized by the complexity of the repairs they make, while the substantive complexity of women's housework is characterized by the complexity of a wide variety of tasks they perform, one of the most important being the complexity of their sewing.

Having created these indices, the investigators are now developing causal models of the relationships between housework and psychological functioning. The initial analyses have been limited to women; they have examined the relationship between women's housework and their intellectual flexibility. The results thus far indicate that women's housework has a profound effect on their intellectual flexibility, the substantive complexity of housework and the closeness with which it is supervised having even greater effects on intellectual flexibility than do the parallel measures of work conditions in paid employment. These results are preliminary and tentative. Schooler and his colleagues intend to analyze the data for housewives and for working women separately, to assess the psychological effects of housework on men, and to take into account the effects of occupational conditions encountered in paid employment, which have thus far not been statistically controlled. Still, the strength of the initial findings makes it seem probable that the main hypothesis -- that the conditions of work encountered in housework will have psychological effects similar to those of the conditions of work encountered in paid employment -- will be confirmed.

5. The structural imperatives of jobs and their rewards. The basic concern of this facet of Carmi Schooler's research is the nature of the relationship between work and its rewards. Specifically, he asks how the structural imperatives of men's jobs are related to both the objective and subjective rewards the jobs provide. The specific rewards considered here are the job's social standing, the income it provides, the incumbent's assessment of its prestige, his sense of the prestige it is accorded by others, and his subjective social class identification.

The principal structural imperatives of a job can be seen as falling into four types: position in the organizational structure (ownership, bureaucratization, position in the supervisory hierarchy); the conditions determinative of occupational self-direction (closeness of supervision, routinization of the work, substantive complexity); job pressures (time pressure, heaviness, dirtiness, number of hours worked); and job uncertainties (the likelihood of a dramatic change in income, reputation, or position; the frequency of being held responsible for things outside one's control; and the risk of loss of job or business).

Schooler found that the substantive complexity of work is by far the most important determinant of the job's social standing. As a determinant of income, though, substantive complexity is less important; in this case, ownership, absence of close supervision, and high position in the supervisory hierarchy have notable effects.

That ownership, hierarchical position, and closeness of supervision are important determinants of income is congruent with neo-Marxist interpretations, which stress the importance of control over the means of production, both for the distribution of occupational rewards, and also in the formation of social classes. This raises the question, how the individual's relationship to the control of production affects his own perception of his occupational status. Relevant data exist in the 1964 survey, where respondents were asked to identify the social class to which they thought they belonged, and to rank the prestige of their occupation both in terms of the way they themselves evaluate it and the way they believe others do. Of the three structural imperatives of a job linked to control of production -- ownership, hierarchical position, and closeness of supervision -- the most important determinant of the individual's view of his social position is closeness of supervision. However, when all of the structural imperatives of the job are included in the regression equations, the substantive complexity of work is by far the most important determinant of the individual's view of his prestige and his position in the class system.

Several theorists have suggested that other categorizations of occupational status would be more powerful determinants of class identification and income than the linear measures of occupational status usually employed in research in this area. Among the criteria of categorization that have been suggested are: white-collar vs. blue-collar; manual vs. non-manual; a "higher" social class that includes skilled workers vs. a "lower" social class of semi-skilled and unskilled workers (embourgeoisement of the working class); a "higher" social class of professionals, managers, and the like vs. a "lower" social class that includes clerical and lower white-collar workers (proletarianization of the lower middle-class). It is possible to test the effect of these several categorizations by seeing whether including any of them in regression equations for income or class identification either adds to the total explained variance or decreases the regression coefficient for the linear measure of occupational status.

Of these criteria for categorizing workers into social classes, only manual vs. non-manual and blue-collar vs. white-collar (measured literally by asking respondents what type of clothing they wear on the job) have significant independent effects on the individual's conception of his social standing and class status, and even these effects are much smaller than those of the linear measure of occupational status. In terms of affecting income, only working with things has a significant independent effect when included in the regression equation together with occupational status. In short, the usual method of treating occupational statue as a linear variable accounts for the lion′s share of the variation in the subjective and objective rewards that the job provides.

6. The Polish replication. The main purpose of this study is to examine the relationship of social class and occupational conditions to psychological functioning in a socialist society. Specifically, the study attempts to test, in a different economic, political, and cultural context, the central finding of Kohn and Schooler in the U.S. that occupational self-direction plays a crucial role in explaining the impact of social class on parental values, orientation toward self and society, and intellectual flexibility. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, prepared a research proposal, which was submitted in November 1977 to the Polish Academy of Sciences, the sponsor of the research. The final approval of this proposal was obtained in January 1978.

The proposal stipulated that the study would be a partial replication of the Kohn-Schooler U.S. study. Questions pertaining to five groups of variables were directly adopted from their 1964 questionnaire. These five groups are: (a) Occupational self-direction, i.e. the substantive complexity of work, closeness of supervision and routinization of work; (b) Orientations toward society and self, expressed in questions designed to index such dimensions of orientation as authoritarian conservatism, anxiety, self-deprecation, self-confidence, idea-conformity, criteria of morality, trustfulness and stance toward change; (c) Parental values, especially those pertaining to valuation of self- direction or conformity to external authority; (d) Occupational commitment; (e) Perceptual and ideational components of intellectual flexibility.

Three variables -- occupational status, education, and income -- were measured in considerable detail by questions that had been intensively tested in previous Polish studies. Questions on stratificational variables, as well as questions on demographic and background variables, were taken from a Polish methodological publication. In this publication, such questions are presented in a standardized form recommended and approved for use in all sociological surveys sponsored by the Polish Academy of Sciences.

In comparison with the American questionnaire, the Polish version was extended by including several questions about membership in voluntary organizations and social participation. It was expected that certain differences in psychological functioning would be explained by involvement in the workers' self-management movement. On the other hand, the Polish version of the questionnaire does not include sections on such phenomena as use of leisure time, job satisfaction, and values for self.

It was decided that the national sample would consist of 1600 males, aged 21-65, living in urban areas, and presently employed full-time in civilian occupations. From this sample, a subsample of 400 persons was selected for additional psychological testing by means of the Embedded Figures Test and the Draw-A-Person Test. Still another subsample of 400 persons was selected for a reliability study.

The preparation of the replicative part of the questionnaire consisted of four steps: translation of questions from the American questionnaire and selection of the best Polish version of each question; a pilot study; modification of the questionnaire after the pilot study; and testing the final version.

The first version of translation of each question was prepared by Slomczynski during a six-week stay at the Laboratory of Socio-Environmental Studies in 1976. The preparation of this translation involved thorough discussions with members of the Laboratory about the meaning of entire questions and special phrases. These exchanges were especially valuable for translating psychological questions that include colloquial expressions. Such phrases as "going to pieces", "end up causing trouble", "to feel upset", etc., have several equivalents in Polish and selecting the proper one requires knowledge of the intention of the question.

Another difficulty was encountered in finding equivalents for situations that do not arise in Poland. For example, American respondents were asked to answer the question: "Suppose you wanted to open a hamburger stand and there were two locations available. What questions would you consider, in deciding which of the two locations offers a better business opportunity?" In Poland there are no hamburger stands, but certain types of newsstands (kiosks) are very popular. However, the concept of entrepreneurial gain is not applicable. The final version of the Polish question was: "Suppose for a moment that you have to decide where a newsstand should be located in a new apartment development. There are two possible locations and you can choose one of them. What questions would you consider in deciding which of the two locations is better?". For measuring intellectual flexibility, the Polish version of the question seemed to serve its purpose.

In further work, several versions of translation of each question were prepared by graduate students of the English Philology Department at the University of Warsaw. The method involved comparison of all versions by substituting whole phrases or single words in appropriate places within the sentence. The semantic and syntactic adequacy to the English original was judged collectively by the group of experts. The purpose of each question provided by the American researchers was applied as the criterion for selecting the best Polish formulation as equivalent to the original.

The last version of the questionnaire thus obtained was subjected to a pilot study. This study was conducted by interviewing fifty persons selected from extreme groups with respect to education and type of work. The interviewers were experienced research workers, trained to perform the interview and, simultaneously, to take notes on the reception of each question by the respondent. Subsequently, each question was evaluated with respect to the respondents′ understanding of the question and the formal correctness of their answers.

According to the first criterion, the interviewers noted, for each question, whether it had to be repeated or explained. According to the second criterion, the interviewer noted, for each question, whether or not it was formally correctly answered. An incorrect answer could consist of non-acceptance by the respondent of the assumptions of the question. For instance, the agree-disagree question, "Young people should not be allowed to read books that are likely to confuse them," is based on the assumption that such books exist. The respondent may not share this assumption. Alternatively, the respondent may answer within a different conceptual framework than the one required by the question. For instance, the question "How often do you feel depressed?" requires one of the following responses: always, frequently, sometimes, rarely, or never. Instead of using these categories, some respondents could answer "I feel depressed whenever something tragic happens in my life". Formally incorrect answers may also result from a lack of knowledge on the part of the respondent, as evidenced by such responses as "I do not know how to answer"; "It is difficult for me to decide"; "I have no opinion"; and similar responses.

Analysis of the results of the pilot study is contained in a 200-page document. For each question, the frequency distribution was prepared according to both criteria and analyzed by means of a cross-tabulation. A question was modified if 20% of the responses were classified as improper understanding. If the question was cross-classified as improperly understood and was also incorrectly answered by more than 5% of respondents, then this question was modified. In total, about 10% of the questions translated from English were modified at this stage. The version of the questionnaire containing all modifications was again tested on university students and through group discussion among experienced interviewers.

The sample of this study was designed to represent men, aged 21-65, living in urban areas and presently employed full-time in civilian occupations. The size of this population in Poland in 1978 was approximately 6.2 million out of the total population of 35 million.

A three-stage probability sampling scheme was devised. In the first stage, all urban centers were listed and given weights proportional to the population size of these units. Twenty-six urban centers were selected, representing 10% of the population of Poland and 30% of the urban male population. At the second stage, all electoral districts of the selected urban centers were pooled and a random sample of 48 districts was randomly selected. At the third stage, the list of voters was obtained for each selected district and screened for sex and age. From each district, two samples of males, aged 21-65, were randomly selected: the basic sample and the auxiliary sample. The basic sample was the target sample of 1600 cases. It was known, however, that the basic sample would contain cases that would not satisfy the requirement of full-time employment in a civilian occupation. Replacement for such persons, as well as for those who could not be interviewed for other reasons, was obtained from the auxiliary sample. In each district, the size of the auxiliary sample was the same as the size of the basic sample.

The interviewers made 2241 visits to different persons. Among those visited from the basic sample, 442 did not satisfy the criteria of the sample; 114 persons were not reached, and 28 refused to be interviewed. In all, 1557 interviews were completed; 875 persons were from the main sample and 682 from the auxiliary sample.

Evaluation of the quality of the sample is a required document, to be included in the final report of every survey conducted by the Institute of Philosophy and Sociology of the Polish Academy of Sciences. This document is being prepared by the Sampling Group section. One difficulty in evaluating the quality of samples is the absence of an operationally available universe of units of observation. The register of voters contains information on sex and age, but not about occupation; insufficient information in the register of voters necessitates the use of the auxiliary sample. In this situation, however, the analysis of Reports of Failure to Reach the Respondent from the basic sample as contrasted with those from the auxiliary sample, allows one to estimate the sample bias. This analysis has not been yet completed. It has thus far been determined, however, that the sample is not biased with respect to the distribution of age, marital status, number of children aged from 3 to 15, education, occupation and income. For these checks, the data from other samples and official statistics were used.

The Institute of Philosophy and Sociology has a pool of 300 trained interviewers distributed around the country in proportion to the population size of each region and district. From this pool, the 159 interviewers were selected, primarily from locations included in the sample. Interviewers were divided into 15 groups of 10 or 11 persons. In each group an interviewing instructor conducted a two-day training session. After the session, interviewers were examined with respect to their familiarity with the questionnaire. The instructor also evaluated the trial interviews conducted by each interviewer with two persons not included in the sample.

Three months after completion of data-gathering, a reliability study was begun. A sample of 385 respondents, randomly selected, received a letter and a questionnaire from the Institute of Philosophy and Sociology. The letter explained the purpose of the repeated questioning and asked the respondent to fill out and return a brief questionnaire. The questionnaire contained four questions pertaining to the interviewer's performance, and eleven substantive questions taken from the interview schedule.

Fifty percent of respondents replied to the first wave of letters. The non-respondents were identified by the questionnaire number and after three weeks received a second letter. Again, about 50% replied. The remaining group, approximately 90 persons, were approached by interviewers. Ultimately, 321 questionnaires were received, or about 83% of this subsample.

A provisional analysis points to the high quality of the data. For example, all respondents indicated that the interviewer consistently made notes during the interview; no information was obtained that the behavior of interviewers was incorrect or improper. As another example, respondents to the mailed questionnaire were asked to describe their own and their father's occupation and education; a cross-tabulation of these data with those from the personal interview revealed no major differences.

After the data had been coded and edited in Poland, Kazimierz Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Much the work thus far has been devoted to problems of cross-national comparability of indices.

Cross-national survey instruments should be evaluated from two perspectives. First, it must be established that questions developed in one cultural context differentiate respondents similarly in another society. The distribution of responses to particular questions could differ between nations because of inexact translation, the substitution of equivalent but not identical questions, or social and demographic differences that alter the relevance of the questions. Second, the investigators must determine whether sets of questions reflect the same concepts. This issue may be especially problematic in measuring psychological phenomena.

To assess whether the Polish survey had successfully replicated the earlier American survey, Slomczynski, Miller, and Kohn examined the distribution of responses for all the important questions. There were two main concerns: that each question differentiated respondents in the Polish survey, and that the patterns of these responses (or the shape of the distribution) was similar to the one expected. Clearly, the interpretation of particular variables would be influenced by unexpected bimodal distributions, severely truncated scales, or other patterns of responses that suggested that the question meant something different in the Polish context. This examination of the central questions used in the survey showed that the questions -- both those identical to and those equivalent to the American items -- differentiated respondents, that the scales imposed on responses were suitable for the Polish population, and that the distribution of responses generally conformed to expectations. This preliminary assessment increased confidence in the comparability of measurement in the replication study, but did not guarantee that these questions were capturing similar concepts.

The analysis of conceptual comparability focused on the measurement of psychological variables, particularly a battery of 57 questions about social orientation and self-conception that were asked in the U. S. and Polish surveys. Using exploratory factor analysis, the investigators found, with only a few exceptions, that each of the questions is related to the same factors in the Polish as in the American data. Nonetheless, it was necessary to determine which questions were the best indicators of each dimension of social orientation and self-conception in Poland. Three criteria were used. First, a question had to be consistently related to a factor, regardless of the number of factors used in the exploratory factor analysis. Second, the question had to have a high factor loading, as compared to those for other questions, in all the exploratory solutions. Third, the question could not be related to more than one factor. Employing these criteria, the investigators identified subsets of questions that best measured a particular aspect of psychological functioning in the Polish data. This subset of questions was then used in a confirmatory factor analysis to produce a measurement model for the concept. All such measurement models were found (by the chi-squared goodness-of-fit test) to fit the data well.

To evaluate whether these measurement models are indexing the same concepts as those indexed in the U.S. study, another confirmatory factor model of each psychological concept was developed. These models use the questions that best indexed each concept in the exploratory factor analysis of the American data, thereby creating models exactly parallel to those used in the original U.S. study. In every instance, the model tailored to be exactly parallel to the American model fits the Polish data well. Moreover, and of crucial importance, the correlation between the two indices of each concept is in every instance very high --

generally, in the high 0.90′s. The evidence is thus considerable that the concepts measured in the Polish replication are highly similar to those measured in the American study. Correspondingly, a similar procedure applied to the American data -- developing two measurement models of each concept, one suggested by exploratory factor analysis of the American data, the other by exploratory factor analysis of the Polish data -- yields similar results. The underlying concepts reflected in these questions are the same in the two countries.

Using the confirmatory factor-analytic models to create indices of the principle concepts, the investigators have also carried out preliminary analyses addressed to the central questions of the Polish replication: Is the relationship between people′s position in the system of social stratification and their values and orientation similar in socialist Poland as in the capitalist U.S.? If so, is the relationship between stratificational position and values and orientation substantially attributable, in Poland as in the U.S., to occupational conditions, in particular, to the substantive complexity of work, routinization, and closeness of supervision? Tentatively, on the basis of excellent indices but only incomplete and exploratory analyses, the answer to both questions seems to be a very decided, "yes." It would seem that social stratification has much the same psychological impact in the two social systems, and for much the same reasons. But, again, these findings are tentative; much analysis remains to be done before they can be accepted as definitive.

7. The Japanese replication. During the year plans were completed and work begun on another major replication of the occupations study, this one in Japan. This replication is being carried out by Atushi Naoi and Kenichi Tominaga of the Department of Sociology of Tokyo University, in collaboration with Schooler. The replication will involve interviewing a probability sample of more than 800 employed Japanese men, using a questionnaire containing all the questions necessary for indexing the structural imperatives of the job and those aspects of psychological functioning measured in the American and Polish studies. Pretesting of this questionnaire, which was developed by the Japanese investigators after close and extensive consultation with Schooler, has recently been carried out. Present plans call for data collection to take place during the summer of 1979. The data will be coded for statistical analysis in Japan during the 1979-1980 academic year and it is expected that data analysis will begin in September 1980, when Naoi is scheduled to come to the Laboratory as a Visiting Scientist.

8. Social class and parental valuation of self-direction or conformity to external authority. Kohn and Schooler′s original factor analysis of the data on parental values in the 1964 survey found the first dimension to be valuation of self-direction or conformity to external authority: the characteristics that are positively correlated with this factor are an interest in how and why things happen (i.e.,

curiosity), consideration, self-control, good sense and sound judgment, and responsibility; the characteristics that are negatively related to this factor are neatness and cleanliness, good manners, being a good student, and obedience to parents. (The second dimension reflects the age of the child.) The parental-values questions have subsequently been included in several other U.S. studies, notably the National Opinion Research Center's General Social Surveys of 1973, 1975, and 1976. Melvin Kohn and Carrie Schoenbach did factor analyses of fathers' and mothers' values for each of the three NORC surveys. Since NORC asked about children of nonspecified age, there is no reason to look for an "age" dimension in these values. They therefore limited the analysis to a single factor, asking simply whether that factor reflects self-direction/conformity to external authority. Separate analyses of fathers and mothers in all three years confirm unequivocally that it does.

One might wonder whether this confirmation that self-direction/conformity to external authority is a fundamental dimension of U.S. parents' values somehow reflects a particularly American concern with self or individuality or independence. Kohn hypothesized, to the contrary, that self-direction/conformity to external authority is an invariant dimension in parental values in all industrialized societies. Data with which to test this hypothesis have generously been provided by colleagues who have done studies of parental values, modelled on the Kohn-Schooler inquiry, in six disparate countries: Italy, West Germany, Taiwan, Ireland, Poland, and Japan. Although all the investigators made conscientious efforts to translate (and in several of the studies to back-translate) the characteristics accurately, there are undoubted differences in the connotations of the same expressions in different languages. Nevertheless, it should be possible to find a self-direction/conformity dimension in all these sets of characteristics. Kohn's hypothesis -- a very strong hypothesis -- was that the first unrotated factor in all these bodies of data would be a clear self-direction/conformity dimension. In general, this hypothesis was confirmed, although in two studies (one done in Ireland, and one of two done in Japan) he had to go to a two-factor rotated solution to find a clear self-direction/conformity dimension. Still, the principal finding is a striking affirmation of the presence of a self-direction/conformity dimension in parental values in all the countries studied.

The correlation between social class and fathers' valuation of self-direction, in the original Kohn-Schooler study, is 0.34, and it is of roughly similar magnitude in subsequent studies of both fathers' and mothers' values. This correlation is rather substantial, considering that correlations between any single dimension of social structure and psychological phenomena never are very large. After all, there are many other determinants of values and orientation -- idiosyncratic personal experience, situational constraints, cultural and institutional imperatives, and facets of social structure other than class itself. Still, Kohn and Schooler place so much theoretical weight on values as the bridge between social structure and behavior, that it is desirable to re-assess the magnitude of the relationship between social class and parental valuation of self-direction of conformity to external authority, using newer methods of analysis than were available at the time of the original study. Using confirmatory factor analysis, Kohn, Schooler, Schoenbach, and Karen Miller have developed measurement models of parental values for the three studies based on national (as distinct from single-city) samples -- the original Kohn-Schooler study, the NORC General Social Surveys, and the Polish replication. With such models, they found the class-values correlation to be approximately 0.45 - 0.50 for both mothers and fathers. Using the longitudinal data of the Kohn-Schooler follow-up study, which permits a much more precise measurement model of parental values, they found the correlation between social class and fathers′ valuation of self-direction/conformity to be approximately 0.60. This is the most accurate estimate of the actual magnitude of the correlation possible with present methods. It is indeed impressive.

Publications:

Kohn, M. L.: The Effects of Social Class on Parental Values and Practices. In David Reiss and Howard Hoffman (Eds.): The American Family: Dying or Developing, New York, Plenum Press, 1979.

Kohn, M. L., and Schooler, C.: Job Conditions and Intellectual Flexibility: A Longitudinal Assessment of their Reciprocal Effects. In Edgar F. Borgatta and David J. Jackson (Eds.): Factor Analysis and Measurement in Sociological Research: A Multi-Dimension Perspective. California, Sage Publication (In press).

Kohn, M. L.: Personlichkeit, Beruf und Soziale Schichtung. (Peronality, Occupation, and Social Stratification.) (In collaboration with Carmi Schooler and Leonard I. Pearlin, with an Introduction by Kurt Luscher). Stuttgart, Klett (In press)

Miller, J., Schooler, C., Kohn, M. L., and Miller, K.A.: Women and Work: The psychological effects of occupational conditions. Am. J. Soc. 85: 1979.

Miller, J.: Hospital response to the legalization of abortion in New York State: An analysis of program innovation. J. Health & Social Behav. In press.

Honors and Awards:

Melvin L. Kohn was elected a Fellow of the American Academy of Arts and Sciences. He also serves this year as President of the Sociological Research Association.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00674-09 LSES

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Social, Psychological, and Biological Interactions in Schizophrenics and Normals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Schooler	Research Psychologist	LSES	NIMH
Other:	T. Zahn	Research Psychologist	LP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology, NIMH

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This year has seen the extension of this line of investigation through the use of confirmatory factor analysis to explore the nature of psychophysiological arousal during task performance.

PHS-6040
(Rev. 10-76)

Although maximum likelihood confirmatory factor analysis was developed in the context of sociological, economic and psychometric studies, the method may be a particularly suitable approach for examining the patterning of psychophysiological arousal. An experiment designed to deal with this subject had already been carried out in collaboration with Dr. Theodore Zahn of the Laboratory of Psychology and Psychopathology. In this experiment the normal subject's heart rate and skin conductance were continuously measured under conditions of rest, stress, and performance of two tasks. Various measures of skin conductance and heart rate base levels and lability have been derived for each of these periods, as well as separate measures of the physiological responses to the anticipation, perception, response and reinforcement segments of one of the tasks.

Since the confirmatory factor analysis provides chi square estimates of how well different models fit the data, it was possible to compare different models of psychophysiological arousal in terms of how well they described the experimental data.

Our initial confirmatory factor analyses involved the determination of the model which best describes the patterns of those psychophysiological reactions occuring during rest and simple task performance which were not elicited by the demands of the experimental conditions. Four different models were tried: 1) a single-concept model in which heart rate and skin conductance were seen as part of the same reaction pattern; 2) a two-concept model in which heart rate and skin conductance were seen as part of separate reaction patterns; 3) a two-concept model which divided both the heart rate and the skin conductance measures in terms of whether they indexed basal levels or lability; and 4) a three-concept model in which the basal and lability components of the skin conductance are separated and the heart rate indices are considered a single factor. The last model seems to provide the best fit to the data. We are now in the process of refining it and developing similar models for experimentally elicited responses. When this is done we hope to be able to use our data to more clearly define the interrelationships among psychophysiological arousal, psychological functioning and task performance in normals--relationships which must be more clearly understood if we are to make sense out of the various theories about the ways in which the psychophysiological arousal pattern of schizophrenics differs from that of normals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00677-16 LSES

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Health Orientations of Parents and Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	J. Campbell	Research Psychologist	LSES	NIMH
Other:	C. Carney	Psychologist	LSES	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The investigator retired from Government service shortly before the close of Fiscal Year 1978. During Fiscal Year 1979 he has been a Guest Worker in the Laboratory, completing analyses described in previous annual reports and preparing them for publication. There are no new findings to report.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00678-02 LSES

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effects of Industrialization on Family and Sex Role Ideologies of Factory Workers. (Formerly titled, The Effects of Adult Life Conditions on Sex Role Ideologies of Factory Workers.)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI Karen A. Miller	Staff Fellow	LSES	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Socio-Environmental Studies

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the cross-cultural universality, content, interrelatedness, and causal antecedents of family ideologies and women's-rights ideologies of male factory workers in several industrializing societies. Confirmatory factor analyses yielded acceptable measurement models of an ideology about autonomy of the adult from parents and an ideology about extra-familial but not intrafamilial rights of women in India, Bangladesh, and Israel, but not in Argentina, Chile, and Nigeria. These results indicate that there is some, but not universal, cross-cultural regularity in the content of family and women's-rights ideologies. The correlations between the two ideologies are low to moderate; thus they should be treated separately. Path analyses with each ideology as the dependent variable showed differences in direct effects of industrialization variables. Mass media exposure relates negatively to family ideology but positively to women's-rights ideology; education and economic circumstances are important for family but not women's-rights beliefs; job skill level relates to women's-rights but not to family beliefs. When we take into account indirect as well as direct effects, however, a similar set of variables has some sort of effect on both ideologies.

Project Description:

Objectives

The purpose of this study was to examine family and women's-rights ideologies, as reflected in the attitudes of male factory workers in several developing countries, and to explore how these ideologies may be affected by contacts with industrialization. The first objective was to determine the extent to which these two ideologies could be empirically identified in diverse cultures. The second objective was to determine their empirical interrelatedness. The third and primary objective was to determine what factors in the industrialization process, particularly factors associated with the work experience, have independent effects on these ideologies. Aspects of the industrialization process considered were education and intellectual skills acquired, mass media exposure, improved economic circumstances, and several aspects of factory work: skill level, factory-provided benefits, and work with machines. I thus examined how variation in task experiences and other experiences associated with industrialization affect particular aspects of the psychological functioning of workers in countries undergoing rapid social change. In the sense that it bears on the broad theme of occupational effects on psychological functioning, the study is complementary to the broader study of occupational effects on the psychological functioning of U.S. workers, ongoing in the laboratory.

Methods Employed

The data used in the analysis consist of interview responses from about 3800 male factory workers in Argentina, Chile, Bangladesh, India, Israel, and Nigeria. The data were obtained in 1964/65 by the Project on Social and Cultural Aspects of Development, directed by Professor Alex Inkeles, now at Stanford University.

The method involved the following steps. First, I obtained from exploratory factor analysis preliminary information about the internal consistency and cross-cultural comparability of the two ideological constructs. Second, I used this information to construct measurement models of these two constructs, and others for which there were multiple indicators, using the methods of maximum-likelihood confirmatory factor analysis. The third step in the analysis was to obtain correlations among the "true scores". The final step was to input these correlations into multiple regressions, to estimate a path model of the effects on family and women's rights ideologies of a number of experiences associated with modernization.

Major Findings

The exploratory factor analyses of the larger sets of items tapping attitudes about family arrangements and women's rights yielded subsets of items with strong loadings in at least five of the six countries. The family ideology factor was defined by three questions:

a. Should a man or his parents choose his job?
b. Should a woman marry her own or her parents' choice of mate?
c. Should a man care more about his wife or mother?

The women's-rights ideology factor was defined by five questions:

a. Should a young woman be allowed to work away from home?
b. Should men and women receive equal pay for equal work?
c. Would you vote for a woman for high office?
d. Should the sexes work/go to school together?
(Average score on two highly intercorrelated questions.)
e. Has women's freedom been changing too fast?

These indicators were submitted to confirmatory factor analysis, and acceptable measurement models were obtained for both constructs in three countries -- India, Israel, and Bangladesh. These results indicate that there is some cross-cultural regularity in the content of family and women's-rights ideologies, but that the content is by no means universal. In the case of the family, the construct consists of a small but distinct core of attitudes about autonomy of the adult from his or her parents. In the case of women's rights, what emerges is a belief in women's rights in society that is not consistently related to beliefs about sex roles in the home. This finding is consistent with findings from other studies in Taiwan (Olsen, forthcoming) and the U.S. (Mason, et al., 1976) that intra- and extra-familial sex-role attitudes are quite distinct. It is impressive that we find this in other cultures as well.

The second major question of this analysis was how interrelated are the family and women's-rights ideologies. That is, are they empirically independent, or, at the other extreme, can they not even be discussed as separate concepts? To answer this question I obtained, using the LISREL program, correlations between the family and women's rights constructs, and found the following results: India, r=.39, Israel, r=.40, and Bangladesh, r=.11. Thus, there is a low to moderate relationship between the two concepts, with a maximum of sixteen percent of variance shared in any country. From this I concluded that the family ideology and the women's-rights ideology should be treated as separate concepts.

The final and most complex question of the study concerned what aspects of the modernization process are related to family and women's rights ideologies. Independent variables included in the analysis were background variables (age, father's education, mother's education, father's occupation, and urbanness of origin), education and intellectual skills, work experiences (job skill, work with machines, and factory benefits), economic circumstances (income and living standard), and mass media exposure.

I estimated path models in which experiences with industrialization predict each of the ideologies in each of the three countries. When we compare models for the two ideologies, we find several striking differences in what variables affect them directly. The major difference is that mass media exposure relates negatively to family independence ideology and positively to belief in women's rights. In addition, education and economic circumstances are important for family independence beliefs but not for women's rights beliefs. Job skill level is related in more than one country to women's-rights ideology but not to family ideology. When we take into account indirect as well as direct effects, however, a similar set of variables has some sort of effect on both ideologies. Assuming the correctness of our structural model, we conclude that basically the same influences affect the two ideologies in different cultures, but that the exact causal configuration varies considerably for the two ideologies and for different cultures.

Significance of Findings and Proposed Course

This research has contributed to the specification of the cross-cultural universality, content, interrelatedness and causal antecedents of two attitudinal constructs that play an important role in the modernization process. To test causal models requiring fewer assumptions would be desirable, but although reinterview data exists for Israel and Bangladesh, the number of cases in each country (N=100) precludes an adequate test of the complex multivariate model I have developed. Therefore, I consider the analysis with these data completed, and the questions remaining will hopefully be addressed in research with other data.

Publications

Miller, K.A.: The Effects of Modernization on Family and Women's Rights Ideologies: A Cross-National Study. In Bartnicki, A. (Ed.): Proceedings of the Conference on Family and Social Change in the Modern World. Warsaw, Poland, Warsaw University Press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00851-15 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Brain Mechanisms of Species-Typical Behavior in Squirrel Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T.K. Bussard	Biologist	LBEB NIMH
	R.E. Gelhard	Biologist	LBEB NIMH
	C.R. Harbaugh	Animal Caretaker	LBEB NIMH

COOPERATING UNITS (if any)

John D. Newman, NICHD, Behavioral Biology Branch

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.5	2.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study is part of a long-term investigation testing the hypothesis that the striatal complex is basically involved in organizing the expression of species-typical, prosematic (nonverbal) communication. The striatal complex is an evolutionary ancient part of the forebrain comprised of the olfactostriatum, corpus striatum (caudate and putamen), globus pallidus, and satellite collections of gray matter. In previous experiments it was found that lesions of the medial segment of the globus pallidus or interruption of its projections resulted in an elimination or fragmentation of the mirror display of "gothic-type" squirrel monkeys. Recovery of the display might occur after interruption of the pallidothalamic projections, but not the pallidotegmental projections. The present experiments represent a continuing effort to learn whether or not the thalamic or the midbrain tegmentum is more essential for the full expression of the display.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In preceding work it was found that the medial segment of the globus pallidus plays a basic role in the organized expression of a species-typical display of squirrel monkeys. Like the corresponding lateral forebrain bundle in reptiles and birds, the projections from the medial segment divide into a dorsal ascending branch to the thalamus and a ventral descending branch to the thalamic and midbrain tegmentum. It was shown that projections to the ventral thalamus were less crucial to sustaining the display than those to the tegmentum. The present study is part of a continuing effort to learn whether or not the thalamic or the midbrain tegmentum plays a more essential role in the performance of the display. The experiments to be described deal with the effects of lesions in the region of Nucleus tegmenti pedunculopontinus of the midbrain and lesions of the tegmental fasciculus at different levels in the midbrain.

Methods Employed: As in previous years, systematic testing of the mirror display of "gothic-type" monkeys is employed for investigating the behavioral effects of destruction of various cerebral structures (see M-NP-LI-17, 1964). The mirror display is a highly predictable variation of a naturally occurring display used by male squirrel monkeys in the show of aggression, in courtship, and in greeting. Of the five main components of the mirror display, vocalization, thigh-spreading, and thrusting of the erect phallus constitute the so-called trump display. After an animal reaches criterion of 80% or more displays in 30 or more trials, the targeted cerebral structures are destroyed by electrocoagulation, and the monkey is tested for several months. Pre- and postoperative sound spectrograms of vocalization are also recorded.

Major Findings: Since the beginning of the study in 1962, observations have been made on more than 115 monkeys, involving more than 45 "monkey years" of testing. Of the present six operated monkeys, the neuroanatomical examination has been completed on three (L-5, M-5, N-5). All three monkeys were regularly tested for 10 to 11 months following surgery. Last year it was reported that a partial lesion of the Nucleus tegmenti pedunculopontinus had no effect on the performance of the display behavior. One monkey (M-5) of the present series with a bilateral, symmetrical lesion of the tegmental fasciculus and medial part of Nucleus tegmenti pedunculopontinus showed a highly significant deterioration in the performance of the display ($P < 0.001$), manifest at first by a fragmentation of the display (absence of "thigh-spread"). A second monkey (N-5) with a bilateral lesion of the tegmental fasciculus at the level of the third nerve nucleus, together with the destruction of the major part of the red nucleus, showed an early and highly significant decline in the performance of the display ($P < 0.0001$). In a third animal, the symmetrically placed lesions involved part of the thalamic tegmentum and overlying Nucleus parafascicularis at the locus where it is penetrated by the habenulopeduncular tract. This monkey developed a fragmentation of the display, with a highly significant decline in the incidence of the thigh-spread; at the time of sacrifice 10 months later, it performed only sporadic displays ($P < 0.0001$).

One monkey (M-5), which produced normal sound spectrograms preoperatively, uttered no isolation peeps during the postoperative period of testing. Its other vocalizations were normal. Monkey N-5 showed a greatly decreased number of isolation peeps following surgery, but the sound spectrograms were normal in appearance. (Monkey L-5, which failed to vocalize under the special recording conditions before surgery, gave a few low-level isolation peeps in tests during the follow-up observations.)

Significance to Biomedical Research and the Program of the Institute: An analysis of the findings in both the present and preceding experiments indicates that ascending components of the tegmental tract are implicated in species-typical, prosematic communicative displays. The central tegmental tract represents one of the largest and most ancient pathways of the brain stem. In addition to fiber constituents known to be involved in pain mechanisms and motor performance involving olivocerebellar function, it has within the past 20 years been recognized to contain ascending fibers from catecholamine-containing neurons in the medulla, pons, and midbrain.

Proposed Course: To be continued.

Publications:

MacLean, P.D.: Role of Transhypothalamic Pathways in Social Communication. In Morgane P. and Panksepp, J. (Eds.): Handbook of the Hypothalamus, Marcel Dekker, Inc., New York, (in press).

Greenberg, Neil, MacLean, Paul D., and Ferguson, John L.: Role of the paleostriatum in species-typical display behavior of the lizard (*Anolis carolinensis*). Brain Research, 180: 1-13, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00870-03 LBEB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Species-Typical Behavior of Hamsters Deprived from Birth of Neocortex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH
	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T.K. Bussard	Biologist	LBEB NIMH
	S.C. Hamilton	Biologist	LBEB NIMH
	C.R. Harbaugh	Animal Caretaker	LBEB NIMH
	A. Kerlin	Biological Aide	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	0.6	1.15

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present project originally was undertaken to test the hypothesis that the limbic system and striatal complex are sufficient, along with the neuraxis, to give expression to most forms of species-typical behavior. The striatal complex and the limbic system represent two evolutionary ancient systems of the brain, being derived, respectively, from reptiles and lower mammals. It was found that hamsters deprived of the neocortex from birth were capable of giving expression to the usual hamster-typical behavior. In the present experiments the operative procedure prevented the development of both the neocortex and the midline limbic cortex. Immature hamsters with these defects failed to show play-fighting, while adult females exhibited deficits in parental care.

Project Description:

Objectives: The original purpose of the present project was to test the hypothesis that the two older evolutionary formations of the brain (the striatal complex and limbic system) are sufficient, along with the remaining neuraxis, for the expression of most forms of special typical behavior. In line with this prediction, it was found that hamsters deprived from birth of the neocortex grew and developed like normal animals and were capable of the usual hamster-typical behavior, including mating and breeding. The present experiments are concerned with behavioral deficits that occur when, in addition to the neocortex, the operative procedure destroys the midline limbic cortex.

Methods Employed: Behavioral observations are made on Syrian hamsters (Mesocricetus auratus) derived from wild stock captured by M.M. in 1971. For the present experiments, microsurgical techniques were used for removing the neocortex and midline limbic cortex on the second postnatal day. Animals were anesthetized by cooling. After a midline scalp incision calvarial flaps were reflected on each side, and the cortex removed by suction. In the follow-up behavioral observations, littermates served as controls.

In an attempt to devise a less radical operative procedure, experiments are now being conducted in which destruction of the developing cortex is achieved by microinjections of a rapidly acting fixative underneath the calvarium.

Major Findings: Two female (LC2, LC3) and two male hamsters (LC4, LC5) survived and reached maturity. Except for small remnants near the olfactory bulb, there was a complete absence of neocortex. The midline cingulate cortex was absent in all animals, and in all except LC4 the dorsal midline hippocampus was virtually non-existent.

The female LC3 showed some retardation in her early development; the growth curves of the other animals were not notably different from those of the controls.

All four animals showed many hamster-typical behaviors, including seed cracking, food pouching, flank-marking, species preference, and circadian activity. All four, however, failed to block tunnels, while the two males built poor nests and failed to hoard.

LC2, LC3, and LC5 never engaged in play-fighting. A single instance of play-fighting was observed in LC4 on the 15th day. As noted above, this was the single animal in which there was preservation of the dorsal, midline hippocampus.

The two females LC2 and LC3 showed excessive flank marking; they mated, became pregnant, and gave birth, respectively, to 8 and 10 pups. LC3 failed to rear her young, while LC2 showed deficits in pup retrieval.

LC5, the animal with the most extensive loss of brain tissue mated successfully, but showed retardation in achieving ejaculation. LC4 failed to mate despite treatment with testosterone.

All the experimental animals were unusually difficult to handle. The behavior of all was characterized by stereotyped circling or pacing during the dark phase of the light-dark cycle.

DECORTICATE

Significance to Biomedical Research and the Program of the Institute: The striatal complex and limbic system represent ancient evolutionary formations of the mammalian forebrain, reflecting an inheritance, respectively, from reptiles and lower mammals. In preceding work, it was shown that in hamsters deprived from birth of the neocortex, but retaining striatal and limbic structures, are capable of giving expression to the recognized forms of species-typical behavior. There are indications from the present study that with the additional loss of the midline limbic cortex play-fighting may not occur and there may be deficiencies in maternal care of the young. Although this is the first time such combined deficits have been reported, the results might have been expected on the basis of known differences in the behavior and brain structure of reptiles and mammals.

Proposed Course: To be continued.

Publications:

Murphy, M.R.: Behavior Patterns: Hamster. Part I. Development and Control. In Inbred and Genetically Defined Strains of Laboratory Animals, Vol. 2, P.L. Altman and D.D. Katz (Eds.): Federation of American Societies for Experimental Biology, Bethesda, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00874-03 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Neuroethology of Reproductive Behavior: The Role of Endogenous Opiates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

C. Pert and D. Bowie, Biological Psychiatry Branch, NIMH

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.95	.6	.35

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endogenous opiate peptides have been found in an area of the limbic system known to be involved in copulation. In men, use of opiate drugs can cause severe sexual dysfunction. We therefore investigated the role of the endogenous opiates in sexual function by using a convenient animal model to (1) assay for changes in plasma beta-endorphin during copulation, and (2) analyze the effects of opiate agonists and antagonists on mating behavior. Radioimmunoassay of plasma extracts revealed beta-endorphin to be at least 86 times higher in males that had just experienced their fifth ejaculation than in unmated males. Levels following a non-ejaculatory intromission, occurring after the fifth ejaculation, were at least three times higher than those of unmated males. In another experiment, both methadone and naltrexone were found to cause a dose dependent reduction in libido, with the methadone effect being the more severe. Potency (ability to achieve erection and intromission) was greatly reduced by methadone but was significantly improved by naltrexone. Pretreatment with naltrexone blocked the effects of methadone; posttreatment reversed the effects.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this project is to identify forebrain mechanisms of species-typical reproductive behavior. The suggestion that the endogenous opiates may be involved in reproduction comes from three areas: (1) opiate receptors are found in parts of the limbic system known to be involved in copulation; (2) opiate receptors are found in the vas deferens; and (3) opiate use in humans often causes severe sexual dysfunction.

In Experiment I of the present study, assays were obtained of changes in plasma β-endorphin during copulation, and in Experiment II, an analysis was made of the effects of an opiate agonist (methadone) and an opiate antagonist (naltrexone) on the mating behavior of male hamsters.

Methods Employed: Experiment I. Six male hamsters in one group were decapitated 30 seconds after their fifth ejaculation, during the refractory period. Six hamsters in a second group were decapitated 30 seconds after a non-ejaculatory intromission, occurring 3 to 10 minutes after their fifth ejaculation. The six members of a third group were decapitated after removal from their home cage. Blood was collected in EDTA (5ml) and bacitracin (500μg/ml), plasma obtained, and β-endorphin extracted by alumina absorption.

Experiment II. Ten male hamsters were each tested for mating after injections of (1) saline; (2) 1, 2, 4, 8, or 16mg/kg of methadone; (3) 20 mg/kg of naltrexone; or (4) a combination of both drugs. Methadone was administered 30 minutes before testing and naltrexone was administered 60 minutes before testing. Tests of the same animal were at least 5 days apart. Sixteen behavior patterns seen during mating were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency, and average duration of each activity. Only the results of two behavioral measures, illustrating the most salient drug effects, will be presented here. These are: (1) total sexual activity, a measure of general libido; and (2) hit rate, a measure of potency.

Major Findings: Experiment I. Radioimmunoassay of plasma extracts showed non-detectable levels of β-endorphin ($<5\text{x}10^{-13}$M) in the unmated group. β-endorphin levels were at least 86 times higher ($4.3\text{x}10^{-11}$M, $p<.01$, Student's T-test, 2-tailed) in plasma collected following ejaculation. Levels in plasma collected after a non-ejaculatory intromission were slightly, but significantly, elevated ($1.6\text{x}10^{-12}$M, $p<.01$). Levels after non-ejaculatory and ejaculatory intromissions were not significantly different ($p>.05$).

Experiment II. Methadone treatment was found to result in a dose-related decrease in both hit rate and total sexual activity. Hit rate was decreased significantly at the 2.0mg/kg dose, but total mating activity was not decreased significantly until the 8.0mg/kg dose. Thus, sexual potency was more sensitive to the opiate than was sexual motivation. As with methadone, naltrexone treatment resulted in a significant decline in sexual activity; however, in direct contrast to methadone, treatment with naltrexone caused an increase in hit rate. That is, the opiate antagonist increased potency, whereas the opiate agonist decreased it.

When naltrexone was administered before the methadone, the effects of the two drugs on total sexual activity and hit rate canceled. When naltrexone was administered after a methadone test in which no copulation occurred, mating was initiated within 2 minutes. This mutually antagonistic effect of their combination suggests that the two drugs act by different mechanisms to produce the same effect on libido.

Significance to Biomedical Research and the Program of the Institute: Our results indicate a role for the endogenous opiates in male reproductive behavior. The finding that naltrexone facilitates penile erection and intromission suggests that opiate antagonists may be useful in treating some forms of sexual impotence.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00839-02 LBEB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

CNS Structures in Mammal-Like Reptiles. I. Forebrain and Epiphyseal Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
	E.C. Roth	Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

Dr. Nicholas Hotton, III, Dept. of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: .75	PROFESSIONAL: .6	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The early evolution of the mammalian forebrain can be best examined by comparing the progressive changes occurring in brain morphology among ancestral mammal-like reptiles (therapsids). Endocranial brain casts are currently uncommon due to the lack of adequately prepared fossil skulls, their unavailability, and the generally held belief that the walls of the endocranium are poorly ossified. The purpose of this project is to prepare skulls acquired at the Smithsonian Institution for new brain casts using more modern techniques. Initial preparations indicate that the endocranium is complete enough to provide valuable and informative brain casts. In addition, it is clear that both carnivorous and herbivorous therapsids will have to be compared for an evaluation of the impact of life style (solitary vs. gregarious groups) upon brain morphology.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A thorough analysis of the evolution of the third eye conducted at the Smithsonian Institution (1976-1977) has indicated that very little information exists in regard to the evolution of the mammalian forebrain from reptilian ancestors; yet the structures of the mammalian forebrain are usually assumed to reflect neural organization among ancestral mammal-like reptiles. Although mammals are undoubtedly descendants of mammal-like reptiles (therapsids), only certain groups of the latter (cynodont lineage) are sufficiently mammalian in terms of skeletal structure to be considered properly as ancestors of modern mammals. Good endocranial casts of the forebrain of mammal-like reptiles do not exist and only a few hindbrain casts have been reported in the literature; therefore, one of the major interests of the Paleoneurology Wing is to prepare new endocranial casts and examine the conformation of the forebrain in contiguous lineages of mammal-like reptiles ancestral to modern mammals. Because gross brain morphology can be correlated with the evolution of animal adaptations, the evolution of the forebrain during the reptilian-mammalian transition should generally reflect the evolution and acquisition of certain categories of adaptation and behavior. Both carnivorous (cynodont) and herbivorous (anomodont) lineages will be compared to evaluate the impact of differing life styles in related groups upon gross brain morphology (e.g., the cynodont group represents active animals while the anomodont group represents more sedentary animals).

Methods Employed: Well-preserved cranial vaults of certain groups of mammal-like reptiles (e.g., cynodonts, bauriamorphs) selected for their advanced evolution toward mammals have been designated for diamond saw (.010") sectioning by Nicholas Hotton, III, Research Curator, Department of Paleobiology, Smithsonian Institution. Parasagittal sections are made of each skull and the endocranial matrix is removed from the brain space by use of a pneumatic air hammer and a fine drill equipped with carbide burrs. Once fossil skulls are prepared, liquid latex rubber is sequentially applied to the endocranial space, allowed to harden, removed, and heat cured. Some forms of latex are sufficiently flexible so that the entire brain cast can be collapsed and withdrawn through the foramen magnum in skulls too fragile or rare to section with diamond saws. The endocranial cast produced is examined for the presence, size, location, and relationship of major brain structures.

Major Findings: To date, examination of existing brain casts (certain cynodonts and dicynodonts) indicates that the brain case is sufficiently ossified to provide a reasonable interpretation of brain morphology; that the cerebrum of some advanced cynodonts was small, long, and narrow; and that the olfactory bulbs were large. Olfaction, as in living mammals, must have played an important role in prosematic ("nonverbal") behavior. Pineal bodies (100% in all species) and third eyes (>95% in all species) were prevalent in early mammal-like reptiles; the third or parietal eye was lost as these reptiles assumed a mammalian habitus. The genetic loss of the parietal eye, but retention of the pineal body, may have been a cogent evolutionary event in species adaptations to changing climatic trends. The parietal-pineal complex of living reptiles represents the ancestral primitive pattern for all vertebrates. The occurrence of parietal eyes and pineal bodies among all vertebrates can be classified as E-2 (parietal eye and pineal present), E-1 (pineal present), or E-0 (parietal eye and pineal absent). Most mammal-like reptiles have the E-2 complex; however, the nearly mammalian forms have evolved the E-1 condition.

Significance to Biomedical Research and the Program of the Institute: Examination of endocranial brain space casts among mammal-like reptiles should yield significant information leading to a more thorough understanding of the evolution of the mammalian brain. Endocranial casts reflect the temporal acquisition of mammalian brain structure as advanced therapsid reptiles became more mammalian. For example, olfaction plays an important role in mammalian reproduction and communication. The olfactory bulbs in cynodonts are especially large in comparison to living reptiles, suggesting that mammalian olfactory communication was well developed among mammal-like reptiles. In contrast, the cerebral hemispheres are not large in cynodonts and are still reptilian in gross morphology. Existing endocranial casts indicate that the brains of certain mammal-like reptiles share common morphological and perhaps functional similarities with the brains of living mammals.

Proposed Course: To be continued.

Publications:

Roth, J.J. and Roth, E.C.: The Parietal-Pineal Complex among Paleovertebrates: Evidence for Temperature Regulation. In: A Cold Look at the Warm-Blooded Dinosaurs, E.C. Olson and R.D.K. Thomas, eds., West View Press, Boulder, Colo., 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00840-02 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ontogeny of the Epiphyseal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.C. Roth	Staff Fellow	LBEB NIMH
	J.J. Roth	Staff Fellow	LBEB NIMH
Other:	S. Snyder	Biological Aid	LBEB NIMH
	C. Lambert	Biological Aid	LBEB NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS: .65	PROFESSIONAL: .45	OTHER: .2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sequence of events which occur during development of the pineal gland and migration of the parietal eye to the parietal foramen has not been established in lizards. This project records normal development in known-age embryos of the side blotched lizard Uta stansburiana, eastern fence lizard Sceloporus undulatus, sage brush lizard Sceloporus graciosus, and several populations of parthenogenetic and bisexual whiptail lizards (Cnemidophorus). Morphological and histological events form the basis for a reference atlas which marks appropriate times during ontogeny when neurosurgical techniques will be most successful. The embryological origin of parietal eyes will be established and, as experimental sets of animals mature, age related changes in the central nervous system will be recorded.

Project Description:

Objectives: Embryological development of the brain has been studied in some members of the Class Reptilia, but few published reports have dealt adequately with the ontogeny or serial development of the parietal-pineal complex. Questions surrounding the origin of parietal eye and pineal gland anlage or their anatomical connections with deep brain structures (habenular and commissural relations) have not been examined. In the Paleoneurology Wing, more than 200 known age lizard embryos have been incubated in controlled photothermal chambers and examined to assess morphological development of the epiphyseal complex before and after hatching. Since the parietal-pineal complex has been shown to play an integral role in temperature regulation, reproduction, and some circadian rhythms in lizards, this survey will demonstrate not only the origin and development of the epiphyseal complex, but also its anatomical association with other forebrain structures; thus, providing morphological evidence for the structural pathways by which the complex may exert its influence on temperature regulation, physiological modulation, and social interactions. Micrographs of histologic embryonic material form a reference atlas which determines stages in embryological development when neurosurgical techniques may be best utilized. Surgical elimination of the epiphyseal complex in hatchlings and subsequently in adults may illustrate the manner in which embryonic epiphyseal tissues and hormones "program" adult behavior. As known age hatchlings (controls and experimentals) mature, age related anatomical changes in forebrain-epiphyseal relations will be recorded and correlated with behavioral and physiological observations.

Methods Employed: Mass incubation and hatching of reptilian eggs has presented serious problems in the past. With information about specific biophysical characteristics now available, it is possible to culture reptilian eggs successfully. Developing embryos of parthenogenetic (unisexual) and bisexual lizards are examined histologically with routine paraffin procedures. Ultrastructure of developing pineal and parietal eye tissues and their relationships with other forebrain structures are being examined to assess maturational changes. The projections of the pineal and parietal eye will be demonstrated by utilizing autoradiograph techniques including H^3-thymidine and H^3-proline tracers.

Major Findings: Histologic sections are photographed and compiled in a reference atlas for neurosurgical techniques. The optimal period for surgical intervention in epiphyseal development has been determined to be 35 days after incubation begins. The parietal eye apparently migrates rostrally in the cranium to a midsaggital position above the cerebrum and in the parietal bones. A very large fontanelle similar to the parietal fontanelle in mammals is common in lizard hatchlings. As animals grow, the area around the parietal eye begins to ossify; there is evidence suggesting that parietal foramen closure in mature adults correlates with cessation of reproductive activity, perhaps through natural destruction of the parietal eye. Experimental destruction of parietal eyes leads to an alteration in temperature regulation which subsequently has similar retardant effects on reproduction. The pineal gland is attached to the parietal eye by a long, forward extension and may make neural contact with the habenular nucleus and posterior commissure in _Anolis carolinensis_. The lumen of the pineal is lined with photoreceptive cells similar to those of the retina of the parietal eye.

Significance to Biomedical Research and the Program of the Institute: This project provides a neuroanatomical basis for timing surgical intervention in a series of experiments in this Laboratory that are designed to examine the role of the epiphyseal complex in temperature regulation, physiological control, and behavioral interactions in ectothermic lizards. The embryological origin and dating of photosensory and secretory epiphyseal systems of lizards will be helpful in reaching a better understanding of the age related changes observed in other vertebrates with secretory pineals (birds and mammals).

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00841-02 LBEB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Physiological and Ethological Role of the Epiphyseal Complex in Ectothermic Vertebrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
	E.C. Roth	Staff Fellow	LBEB NIMH
Other:	S. Snyder	Biological Aid	LBEB NIMH
	C. Lambert	Biological Aid	LBEB NIMH
	H. Page	Biological Aid	LBEB NIMH

COOPERATING UNITS (if any)

Dr. William A. Gern, Dept. of Physiology and Zoology, University of Wyoming, Laramie, Wyoming; Dr. Elliott Jacobson, Dept. of Laboratory Animal and Wildlife Medicine, U. of Florida, Gainesville, Florida

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.5	.7

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In ectotherms, temperature is important as an environmental cue and as a rate-limiting factor in controlling all aspects of reptilian life processes. The parietal eye and pineal body (E-2 epiphyseal system) participate in the control and selection of optimal environmental temperatures and, thus, secondarily affect reptilian physiology and behavior. This project examines the levels of body temperature which permit the expression of species-typical behavior and physiology (reproduction) in animals lesioned during and after neonatal development. The presence of melatonin in alligators which lack pineals raises the possibility that melatonin is not characteristic of pineals and that pineal bodies do not fulfill the definition of an endocrine gland.

Project Description:

Objectives: Initial experiments have shown that the epiphyseal complex is intimately involved in the control of temperature selection in certain lizards. Since ectothermic vertebrates are dependent upon external heat sources for the energy to be active, temperature control is a major factor that permeates all facets of reptilian physiology and behavior, i.e., metabolism, reproduction, neuroendocrine interactions, and all social interactions involved in reptilian life cycles. One of the hormones (melatonin) produced by the pineal gland has been shown to be an agent that affects temperature selection and hence, reproduction and metabolism. To examine the role of the epiphyseal complex in reptilian life cycles one may utilize surgical manipulations in lizard embryos to interrupt the influence of all or part of the complex before hormonal imprinting occurs. When the embryonically deprogrammed animals achieve maturity, both physiological and ethological alterations of the normal life cycle will be compared with control animals.

1. Physiology - Body temperature control, reproductive patterns, and endocrine physiology are being examined.

2. Ethology - Ethograms are being developed for an ontogenetic sequence of known-age animals.

Methods Employed: In adult animals, surgical extirpation of a pineal body is accomplished through a cranial aperture created by a small dental burr (1 mm). In embryonic lizards, the timing of extirpation of the pineal usually occurs on or about the 35th day (see Project No. 00840-02).

1. Physiology - Body temperatures are being recorded by two 24-channel Honeywell multipotentiometers and reproduction is being examined by specific assays for female lizards (number and size of oocytes; presence of corpora lutea; stage of reproductive cycle; and endocrine condition). Hormonal assays for melatonin have been conducted by Dr. William A. Gern of the University of Wyoming.

2. Ethology - Ethological data are gathered and condensed into ethograms for each species and for age groups within each species. Experimental effects of pineal extirpation are verified by comparing normal ethograms of intact lizards with those of experimental animals. Body temperature data and behavioral inventories are correlated to determine the body temperatures at which species-typical displays are most successful in a conspecific communication (e.g., does the dominant animal in a display remain dominant because his display is performed at or near the most optimal temperature for the information exchange involved in the display). Also, the body temperature of adult animals are compared with behavioral display frequencies to determine the temperature ranges in which species-typical behavior occurs.

Major Findings: 1. Physiology - Reptilian eggs are delivered by unisexual and bisexual parietal-eyed and eyeless species of lizards. The period of incubation (45-80 days) depends on the temperature of incubation and the length of the warm phase within each 24-hour thermoperiod. With a $30^{\circ}C$ photophase and a $25^{\circ}C$ scotophase cycle, eggs from all species hatch in 58-62 days. Previous results indicate female Cnemidophorus velox and C. tesselatus have an oviposition cycle with peaks of oviposition between 2000 and 2400 hours. The peaks of oviposition in an arboreal species, Anolis carolinensis, have a bimodal distribution of 1300 and 2100 hours. Both Cnemidophorus and Anolis are ground nesting lizards, but the former spend the night burrowed in the ground and the latter rest in trees. Endocrine events (oviducal contraction after oxytocin stimulation) apparently cycle in these two species so that oviposition coincides with suitable nesting sites. The E-2 complex (parietal-pineal complex) controls the rate of ovarian growth. Parietalectomy retards previtellogenic ovarian follicular growth in the early phases of ovarian recrudescence and only retards ovarian growth in the later phases of ovarian recrudescence if animals are prevented from freely regulating body temperature.

Virtually all modern vertebrates possess some portion of the parietal-pineal complex except crocodilians, anteaters (edentates), and dugongs (marine mammals). If pineal bodies and the pineal hormone melatonin are integral components of neuroanatomical systems affecting temperature regulation, physiology and reproduction, it is important to ask how some vertebrates can function without the pineal or its hormones. Serum from juvenile alligators was assayed for melatonin. This procedure was carried out because (i) the alligator is a member of the Class Reptilia that does not possess an epiphyseal complex; (ii) alligators are nocturnal and active when titres of pineal melatonin (a potential activity suppressant) are high in animals with pineals; and (iii) the Order Crocodilia is the last modern representative of the large subclass Archosauria to which dinosaurs belong. Although pineal bodies are absent in alligators, pineal melatonin (unrelated to a circadian rhythm) was found in the serum (125 picamoles/ml serum). Alligators apparently create melatonin from extra-pineal sources (harderian gland) that produce chronic low level amounts in each 24-hour (circadian) cycle.

The findings suggest that melatonin is not endemic to pineal bodies and therefore that the pineal does not fulfill the definition of a gland since it does not possess a characteristic pineal hormone as has been previously believed.

2. Ethology - Ethological comparison of embryonically epiphysectomized (pineal-less) animals begins upon hatching.

A sequence of naturally occurring behaviors have been observed in a group of lizards hatched in the laboratory. These are now being compared to ethograms generated for wild-captured hatchling, juvenile, and adult parietal-eyed and eye-less species of lizards. For example, free-living iguanids such as Sceloporus undulatus select high perches from which long-range visual displays (assertive and challenge displays) are used to communicate possession of that territory or space by the resident male. These displays can be used to maintain an effective personal distance before confrontation. On the other hand, ground dwelling species such as Cnemidophorus velox do not use elevated perches to convey intraspecific information and so may frequently encounter conspecifics in their habitat. Personal distance in these lizards is maintained without the aid of territories resulting from effective utilization of species-specific displays (e.g., iguanid head bobbing and dewlap extension), but is preserved by a rapid exchange of forward head thrusts, followed by retreat of the submissive animal. If an encounter involves an intrusion on the narrow (for lizards) personal space of the dominant animal, leg-nipping and biting ensue, followed by a rapid retreat of the sub-dominant animal. If the distance between C. velox involved in confrontation is large enough not to violate personal space, both animals freeze in an alert position, view each other, and soon move in opposite directions. Thus, conflicts between lizards of a species may be avoided by the informational exchange transferred by long-range visual displays in arboreal or perching species (e.g., iguanids) or by precise maintenance of personal distance in strictly terrestrial species (e.g., Cnemidophorus). Information such as this will be utilized in further experimentation to evaluate the effects of E-2 and habenular lesions on the normal expression of individual and group behavior.

Significance to Biomedical Research and the Program of the Institute: Information about the effects of the epiphyseal complex on temperature regulation, behavior, and reproduction are essential to a better understanding of the ultimate role of striatal and limbic structures in prosematic ("nonverbal") and other communicative behaviors in reptiles and mammals. In addition, this project will demonstrate the effects of embryonic hormonal imprinting on the programming sequence of adult stereotypic behavior. Evolutionarily, the stability of the pineal hormone melatonin among all vertebrate classes can be contrasted with the genetic loss of the pineal gland proper. Apparently, the gland may be expendable but the pineal hormones are indispensable.

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00842-02 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparative Neuroanatomy of the Parietal-Pineal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.C. Roth	Staff Fellow	LBEB NIMH
	J.J. Roth	Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.45	.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current information on the gross and microscopic anatomy of the parietal-pineal complex among lizards is scarce and inaccurate. This project examines the anatomy of pineal systems and their neural connections with the forebrain in a comparative survey including naturally occurring parietal eyed and eyeless forms. Preliminary analysis indicates that some embryos of parietal-eyeless lizards also lack lateral eyes; these animals also appear to have genetically abnormal pineal bodies. Gross anatomical characteristics of the pineal body vary from what has been described in other published reports in the degree of development and amount of physical contact with the parietal eye. Existing errors in the literature derive from inadequate preparation of tissues and sampling techniques which do not reflect the normal histology or morphology of the majority of animals in any given species.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Both the human pineal gland and other mammalian pineals are often presented in published reports and textbooks as representing the vertebrate pineal in both morphology and physiology. Actually, the mammalian pineal is but one part of a complex of neural structures, including a third eye, which find their fullest development among subavian vertebrates and especially reptiles. Even among reptiles, inadequate sampling and imprecise analysis have led to a confusing synopsis of supposedly normal pineal morphology. The Paleoneurology Wing is examining both the morphological and histological range of neuroanatomical types of pineal bodies among reptiles. Since secretory pineal systems comparable to those of mammals have evolved among certain lizards, a survey of pineal systems among lizards can be used to compare and derive models for the evolution of the mammalian pineal system and to expand the information for reptilian epiphyseal systems.

Methods Employed: The brains of lizards representing naturally occurring parietal-eyed and eyeless species are being examined histologically with routine paraffin methods to determine the amount of variability to be expected in morphology from individual to individual. Afferent connections of the mature parietal eye and pineal body are being examined using horseradish peroxidase and cobalt chloride injections. The gelatin embedding technique is being modified to accommodate a decalcification requirement when a complete skull must be sectioned. The standard Weil Stain has been modified to permit histochemical staining of paraffin embedded material. Ultrastructural examination of pineal tissues from different lizard species is being conducted to differentiate secretory (pinealocytes) and nonsecretory (photosensitive) pineal cells. Members of the reptilian order Crocodilia which do not possess pineal bodies but which do have the hormone melatonin, which is secreted principally by the pineal gland in other vertebrates, are being examined for the presence of residual or vestigal pineal neural tracts.

Major Findings: The survey of the epiphyseal system of the lizard *Anolis carolinensis* indicates that the pineal body connects to the parietal eye in all animals examined. These data differ dramatically from published reports indicating that the Anole pineal and parietal eye are anatomically separate structures. Because the forward pineal extension maximizes exposure of photoreceptive pineal cells by increasing a horizontal instead of vertical exposure to transmitted cranial light, the pineal system of *A. carolinensis* may be neuroanatomically more functional than previously reported. The extensive survey of epiphyseal systems conducted in this research project will generate a new classification of reptilian epiphyseal systems that will revise current published data that are partially in error due to inappropriate sampling techniques.

Although not noted in the literature, ossification of the cranial parietal foramen follows a predictable sequence in animals of increasing snout-vent length (larger and presumably older animals). If the ossification of the parietal foramen in lab-reared animals of known age corresponds to that in increasingly larger animals, a tool will be available for estimating not only age in modern parietal-eyed species of reptiles, but also fossil mammal-like reptiles with parietal eyes. Growth stages of one fossil mammal-like reptile (Daptocephalus) indicate that parietal foramen closure occurred as maturity was reached in this species. Since fossil vertebrates representing different growth stages of a species have been considered to be separate species, this information may make the determination of evolutional sequences more feasible in the lineage of reptiles ancestral to mammals.

The allometric relationship between the brain and its endocranial space is being examined in lizards and in crocodilians as they mature. Preliminary results indicate that the brain fills approximately 85% of the endocranial space in young crocodilians and 70-80% in young lizards. These data differ from reports in the literature that conservatively estimate the relationship as 50%. The importance of this finding is that endocranial casts may be far more informative than is currently assumed since casts of the endocranial space can closely approximate brain structures (forebrain and epiphyseal complex). In addition, existing encephalization quotients may be extremely conservative and misleading for many groups of fossil reptiles.

In three animals, horseradish peroxidase injection into the parietal eye of Anolis carolinensis led to precipitation in the pineal body and the area of the habenula and the posterior commissure. This morphological relationship lends support to electrophysiological studies that have demonstrated transmission of electrical impulses between the parietal eye and pineal. Transport of horseradish peroxidase molecules from the parietal eye to deep brain structures may clarify the function of the epiphyseal complex in the neurophysiology of these ectothermic vertebrates. The histology of five abnormal lizard embryos which died at hatching and lacked lateral eyes showed a minimally developed pineal body, perhaps suggesting a functional or trophic relationship between the ontogeny of the visual system and the photo-responsive pineal gland.

Significance to Biomedical Research and the Program of the Institute: The different phases of this project are important in revealing the evolution of mammalian pineal systems and are fundamental in establishing a framework for assessing normal pineal morphology and histology in modern reptiles. Findings can be applied to modern and fossil vertebrates, thus helping to identify similarities in brain morphology between ancestral mammal-like reptiles and their descendants, the mammals.

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00844-01 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Detection of Proteins in Two-Dimensional Polyacrylamide Electrophoretic Gels: A Highly Sensitive Silver Stain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)
Dr. Carl R. Merril, Laboratory of General and Comparative Biochemistry, NIMH

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

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TOTAL MANYEARS: .6	PROFESSIONAL: .6	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The separation of complex proteins by two-dimensional electrophoresis on polyacrylamide gels is an important analytical method for detecting missing or aberrant proteins. Such proteins may be associated with various genetic diseases including degenerative diseases of the nervous system such as Huntington's chorea. Without the use of radioactive isotopes, visualization of the proteins in the gels is only possible with the use of certain dyes which are less sensitive than the autoradiographic method. By employing a modified procedure of the de Olmos cupric-silver technique, the proteins can be detected in amounts as small as 100 pico grams/mm^2, or about 0.1 picomoles/mm^2--a 100 fold improvement over the sensitivity of the dye method and comparable to what is achieved with autoradiography. With this cupric-silver method, the proteins in human cerebrospinal fluid have been visualized--some of the minor components for the first time.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: The high affinity of the reduced silver method for products of degeneration in the central nervous system as well as for the randomly associated amino acid constituent of gelatin led me to apply these methods for the visualization of proteins which had been electrophoretically separated in polyacrylamide gels. Currently, the dye Coomassie Blue is used to detect proteins in polyacrylamide gels, but its sensitivity is well below that obtained with an autoradiogram of a gel containing proteins labeled with radioisotopes. To label the proteins, cells must be cultured in the presence of amino acids labeled with radioisotopes. Since human material such as spinal fluid, blood plasma, and amnionic fluid cannot be labeled in this way, another method of detection of equal sensitivity would be invaluable. The silver stain appeared to be such a method.

Due to the promising results of initial trials using the cupric silver method of de Olmos, subsequent experiments were directed to achieve control of the staining reaction so that minute quantities of proteins would be revealed but without an obscuring, dark background. Measures were also taken to eliminate those steps of the procedure which were unnecessary for gels, although required for brain sections.

Upon achieving an optimal procedure, silver stained gels containing known quantities of several proteins labeled with radioactive isotopes were compared with their autoradiogram and with a similar gel stained with Coomassie Brilliant Blue.

Methods Employed: Proteins were separated in the first dimension by isoelectric focussing and then by molecular weight on polyacrylamide gels for the second dimension. The overall procedure followed that of O'Farrell (1975). Autoradiograms and gels stained with Coomassie Blue were obtained by standard procedures.

Silver stain. The separated proteins in the gels were "fixed" in place by treatment with a methanol-acetic acid solution immediately upon removal of the gels from the electrophoretic apparatus. Following treatment in paraformaldehyde, the gels were incubated in a cupric nitrate-silver nitrate solution and then an ammoniacal silver solution. The silver nuclei established by these two steps were amplified by the action of a dilute formaldehyde solution which reduces ionic silver to a black metallic form. Optimal times and concentrations of this reduction step were established to insure visualization of minor protein components without an obscuringly dark background.

Major Findings: The sensitivity of the silver stain is at least 100 times greater than that of Coomassie Blue and is comparable to autoradiography for visualizing proteins in polyacrylamide gels. Proteins present in quantities of 100 picograms/mm^2 of 0.1 picomoles/mm^2 (that is 2-3 molecules per cubic micron!) could be seen. Silver stained gels containing samples of human cerebrospinal fluid, amnionic fluid, or urine revealed proteins not visible by staining with Coomassie Blue.

Significance to Biomedical Research and the Program of the Institute: This silver stain for proteins separated by two dimensional gel electrophoresis has numerous important applications. Since all tissue and fluid can be directly analyzed, anomalous or absent proteins associated with various disease states can be detected. The sensitivity of the silver stain allows even the minor protein components in urine, cerebrospinal fluid, and amnionic fluid to be registered. Previously, such fluids could not be analyzed with the required detail, if at all, due to the inability to incorporate radioisotopes into the constituent proteins. (The presence of such labeled proteins in the gel is revealed by autoradiography.) Furthermore, studies to determine the registry of proteins in cell systems with rapidly changing metabolism will no longer be limited in temporal resolution by the amount of time necessary for culturing and incorporation of isotopes. Without these time constraints, for example, changes in protein composition of cerebrospinal fluid and plasma may be correlated with diurnal and ultradian rhythms and may be linked to normal and abnormal emotional states.

The silver stain also poses some advantages to the logistics of research on identifying marker proteins for various disease states. Both time and expense would be reduced for a given series of experiments. A further consequence of these savings is that more investigators can join in this research. The elimination of the necessity of tissue culturing alone can put this kind of research within reach of many more laboratories.

Proposed Course: Preparation has begun in this Laboratory to characterize regions of the brain by two dimensional gel electrophoresis of micropunch samples taken from brain slices. Having achieved a series of "normal protein maps", developmental, aging, and pharmacological analyses can begin.

Publications:

Switzer, R.C., Merril, C.R. and Shiffrin, S.: A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Analytical Biochemistry, in press.

Merril, C.R., Switzer, R.C. and VanKeuren, M.L.: Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. of Nat'l. Acad. Sci., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00845-01 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparative Anatomy and Histochemical Characterization of the Islands of Calleja

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Hill	Biologist	LBEB NIMH
Other:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: .4	PROFESSIONAL: .35	OTHER: .05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to examine the comparative anatomy, histochemistry, and connections of the islands of Calleja of the olfactory tubercle. Analysis to date has shown that the islands of Calleja have an intimate spatial relationship, as well as similarities in histochemistry, with both the ventral pallidum and striatum, suggesting a role for the islands of Calleja in striato-pallidal function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The islands of Calleja are prominent groups of very small nerve cells (granule cells) within the olfactory tubercle of mammals. The estrogen binding capacity of the islands in rats suggests a role in reproduction; the presence of a cilium within each granule cell suggests a sensory role. The present project is designed to provide basic information regarding the function of the islands of Calleja through examination of their comparative anatomy, histochemistry, and connections.

Methods Employed: Experimental work is primarily on the rat, but comparative studies include representatives of several orders of mammals. Nissl and fiber stained 40 μm serial sections of the olfactory tubercle are examined with light microscope. The relationship of the islands of Calleja to the vasculature is being examined by intracardial perfusion with India ink which makes the entire cerebral vasculature apparent. Special histochemical tests are used for localizing acetylcholinesterase, neurosecretory material, iron, succinic dehydrogenase, and catecholamines. Because of the high iron content of the globus pallidus, Perl's method for iron is used to identify the pallidal component of the olfactory tubercle. The connections of the islands of Calleja are studied by two methods: (i) the cupric-silver technique and (ii) the tract tracing method of horseradish peroxidase.

Major Findings: Analysis to date has demonstrated that the islands of Calleja have not only a close spatial relationship with both the striatum and globus pallidus, but also share the iron concentrating feature of the globus pallidus and, like the striatum, contain acetylcholinesterase. Ironpositive, finger-like formations of the ventral pallidum extend rostrally from the caudal polymorph zone of the olfactory tubercle. Each formation is capped by an island. The iron-positive pallidal-like zone extends into the cell-sparse cores of the islands. The neuropil around the granule cells of the islands is heavily laden with iron.

The striatal component of the olfactory tubercle, as revealed by acetylcholinesterase stained preparations, is distinct from the iron-positive areas and extends from the superficial strata through the cell bridges of Heimer to join the main body of the striatum. The islands are intensely stained in these preparations and lie within the acetylcholinesterase-positive area of the olfactory tubercle.

The islands have no "special" relationship with the vasculature nor are they neurosecretory. The determination of the connections of the islands of Calleja with other brain areas is in progress.

Significance to Biomedical Research and the Program of the Institute: The present study will provide the necessary basis for determining the role of the islands of Calleja in nervous system function. The islands share characteristics with both the globus pallidus and the striatum. They are related to the globus pallidus in that they (i) are iron-positive, (ii) are joined to finger-like projections of the pallidum, and (iii) contain pallidal-like tissue within cell-sparse zones. At the same time the islands, like striatum, (i) are intensely acetylcholinesterase-positive and (ii) lie within the striatal component of the olfactory tubercle.

The intimate spatial relationship with the ventral pallidum and striatum and the similarities in histochemistry to both of these areas, suggests a specialized role for the islands in striato-pallidal functions.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00846-01 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Ovarian Hormones on Iron Concentrating Areas of the Telencephalon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Hill	Biologist	LBEB NIMH
Other:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

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Laboratory of Brain Evolution and Behavior

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TOTAL MANYEARS: .5	PROFESSIONAL: .35	OTHER: .15

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ovarian hormones have been shown to influence the levels of hepatic and serum iron. The effect of these hormones on iron in the brain has not been reported. This project examines the effect of manipulating ovarian hormone levels on iron concentrating areas of the telencephalon of male and female rats. Preliminary histochemical analysis indicates that both estrogen and progesterone increase brain iron.

Project Description:

Objectives: The purpose of this study is to determine the influence of ovarian hormones on the iron stores of the brain. A sex difference in iron metabolism has been reported in many species of vertebrates. In rats of both sexes, the concentration of iron in the liver increases with age; however, the female has over twice the amount of liver iron as the male, and this sex difference disappears with removal of the ovaries and testes. Anemic female rats utilize dietary iron more efficiently than anemic male rats. The sex difference in iron metabolism is due to the ovarian hormones estrogen and progesterone. Estrogen increases serum iron by stimulating the synthesis of ceruloplasmin, a ferroxidase. In addition, the synthesis of ferritin is affected by estrogen; the estrogen-induced higher levels of serum iron stimulate the synthesis of ferritin. Progesterone also influences serum iron, although the mechanism by which it acts has not been determined. During pregnancy, both the level of serum iron and the rate of iron uptake by the intestinal mucosa are increased.

Iron deposits are found in many areas of the brain and, in humans, the amount of iron in these areas increases with age, reaching maximum levels at about age 30. The globus pallidus and substantia nigra have the largest stores, with the globus pallidus reaching concentrations comparable to that of the liver. The function of this large deposit of iron in the globus pallidus has not been determined, nor has any evidence been reported that brain iron is affected by ovarian hormones.

Methods Employed: Because of their high iron content, the globus pallidus, ventral pallidum, and islands of Calleja are the focus of the study. Varying levels of hormones are obtained through 1) the normal fluctuation of estrogen and progesterone occurring in the estrous cycle and pregnancy of rats and 2) the effects of exogenous hormones (estrogen implants and progesterone injections) administered to rats of both sexes from which the ovaries or testes have been removed.

The presence of ferric iron is determined by Perl's histochemical method that renders visible only that part of the iron not in combination with heme compounds or masked by ferritin. The intensity of color obtained by the histochemical methods for iron shows a high correlation with the amount of iron as determined by spectrophotometric methods. Perl's technique is used on 50 μm sections of brain tissue.

Major Findings: Preliminary results obtained with Perl's histochemical test for iron show that 32-week-old, pregnant females and ovariectomized females with estrogen implants or progesterone injections have a more intense iron reaction in the globus pallidus, ventral pallidum, and islands of Calleja than ovariectomized females. Thirty-two-week-old male rats, whether controls, castrated males, or castrated males with estrogen implants have a less intense iron reaction than any of the above mentioned female groups. However, among males, those treated with estrogen have the strongest reaction.

Significance to Biomedical Research and the Program of the Institute: Iron deficiency is a prevalent nutritional disorder in human beings. Affected individuals frequently exhibit behavioral symptoms including irritability, fatigue, and (in adolescence) decreased quality of scholastic performance. However, little data is available concerning the effects of iron metabolism on brain functions. Our preliminary results show that the iron stores of the brain are influenced by the levels of ovarian hormones and that females have more brain iron than males. This appears to be the first report of a sex difference in brain iron. It is assumed that the sex difference in iron metabolism is related to the demands of pregnancy and the necessity for a supply of iron for the developing fetus. The sex hormone effect on cerebral iron is possibly implicated in the physiology and behavior of reproduction.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00871-03 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparative Histochemical Analysis of the Ventral Striatal Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH
Other:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.15	.45

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The presence of a ventral component of globus pallidus in the olfactory tubercle was suggested by histochemical, hodological, and electron microscopic evidence. The presence of certain molecular markers, especially iron, in the more dorsal neo-pallidum led to the hypothesis that the purported ventral pallidum in the olfactory tubercle should exhibit the same features. Refinements in the Perl's method for detecting ferric-iron allowed iron to be visualized in all the areas of rat brain previously reported for human brain. The iron-positive zone of the ventral pallidum was seen to extend into the olfactory tubercle of rat, hamster, squirrel monkey, and opossum. These observations together with the reports of high levels of other pallidal molecular markers, the neurotransmitter GABA, and its synthesizing enzyme glutamic acid decarboxylase, provide a strong argument for the presence of a pallidal component in the olfactory tubercle. Phylogenetically, this component may be the primordial or paleo-pallidum.

Project Description:

Objectives: The high content of iron in the main body of the globus pallidus provided a criterion for establishing the ventral boundaries of pallidum. By employing a histochemical method for iron, we seek to determine whether ventral pallidum extends into the olfactory tubercle as other evidence has suggested.

Methods Employed: Perl's or the Turnbull Blue method was used in early studies to determine the location of iron in brains from humans. Contemporary reports using these methods on non-primate brains described the reaction product as weak, thus allowing indistinct localization of the iron. To test our hypothesis of a ventral extension of globus pallidus in the olfactory tubercle (see Z01 MH 00871-02 LBEB, FY 78), the iron in the main or dorsal part of globus pallidus needed to be distinctly visualized. It was therefore necessary to determine the conditions which would yield an optimal reaction using the Perl's method for ferric iron. Once this was achieved, the purported pallidal areas of the olfactory tubercle could be compared with the main body of pallidum.

Major Findings: An intense Prussian Blue reaction product, indicating the presence of ferric iron, was achieved through incubation of the uncut brain in an alcohol-formalin mixture and later reacting sections of the brain at 56°C in the Perl's solution.

Macroscopically, the entire globus pallidus is highlighted by the Prussian Blue reaction product. In parasagittal sections there is a rostro-ventral cusp shaped extension of globus pallidus coinciding with the locus of pallidal-like neurons seen in the Nissl-stained sections. The blue zone extends above the horizontal limb of the diagonal band of Broca to include the rostral substantia innominata and joins the caudal olfactory tubercle in the polymorph layer. More rostrally, the iron-positive zone is divided by the cell bridges joining the ventral striatum above with the superficial layers of olfactory tubercle below. Microscopically, the large multipolar neurons of globus pallidus and of the rostro-ventral extension are embedded in a blue matrix of the neuropil populated by glia whose cytoplasm is stained intensely blue. Occasionally some of the fine processes of the glia are revealed by the Prussian Blue. Compared with the globus pallidus, the iron-positive glia in the caudate-putamen are sparse and the blue neuropil is absent. The boundary between the blue of pallidum and adjacent structures is distinct; even the narrow, striatal cell bridges of the olfactory tubercle are iron-negative despite the intense blue of the surrounding polymorph layer.

Taken together, the iron-positive glia and neuropil form a continuum which consolidates the telencephalic projection areas of the caudate-putamen, accumbens, and the striatal cells of olfactory tubercle. These findings complement those of Heimer and Wilson regarding the striatal character of components of olfactory tubercle and, in light of the high GABA and GAD levels found in the polymorph layer, provide a strong argument for a pallidal component in olfactory tubercle--perhaps more correctly termed "paleo-pallidum".

Other iron-positive areas of the brain are: ventral-lateral thalamus, medial-dorsomedial nucleus of thalamus, substantia nigra - pars reticulata, interpeduncular nucleus, cerebellar nuclei, inferior colliculus, lateral lemniscus, superior olive, facial nucleus, and pineal body.

Significance to Biomedical Research and the Program of the Institute: The presence of pallidum in the olfactory tubercle calls for an expansion of our thinking of the role of pallidum. Notions that it is merely a motor nucleus have been replaced by consideration of its involvement in the expression of species-typical behavior--behavior is not expressed without a motor event. Pallidum is a massive structure receiving, by way of the caudate and putamen, input from all areas of neocortex and then modulating the ventral ("motor") nuclei of thalamus with its output. This structural sequence is the neocortex/neostriatum/neopallidum/"neo"thalamus system. There appears to be also a "paleo" system: paleocortex/paleo(ventral)-striatum/ and now, paleopallidum/dorsal (paleo?) thalamus. It now seems that pallidum is a common element to both neo- and paleo-cortices.

While there is still much to understand before the function of pallidum is known, these data, by expanding our view of pallidum, may elucidate the principle of operation common to both paleo- and neopallidum. In attaining this level of understanding, we may also progress toward understanding underlying mechanisms to normal behavior states as well as abnormal states such as schizophrenia and depression.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00872-02 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparative Study of Olfactory Projections to Neopallium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The projection of the olfactory bulb in opossum to the neopallial part of insula cortex was established using the cupric-silver method for detecting degeneration. To confirm this projection, horseradish peroxidase (HRP) was injected into the olfactory bulb of opossum. The anterograde movement of the HRP was detected using a new, more sensitive, histochemical procedure. This procedure allowed visualization of the HRP reaction product in axon terminals present in the same areas where degenerating terminals had been found, including the neopallial part of the insula cortex.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: Confirmation of a direct projection to neopallial insula from the olfactory bulb in opossum, established by a degeneration silver stain study, was sought using the horseradish peroxidase tract tracing method.

Method Employed: The olfactory bulb of an opossum was injected via glass micropipette with a solution of horseradish peroxidase (HRP). Following a 24 hour post injection survival, the animal was perfused and the brain sections treated with tetramethylbenzidine and hydrogen peroxide to reveal the presence of HRP. This procedure allows visualization of the synaptic terminals and axons which emanate from the neuron cell bodies within the injection zone.

Major Findings: The HRP-positive axons and terminals occupied the same zones of the opossum neo-pallium and olfactory cortex as were revealed in an earlier degeneration-reduced silver study.

Significance to Biomedical Research and the Program of the Institute: These results confirm that the olfactory bulb of opossum projects directly to the neopallial insula. Other mammals, such as rat and guinea pig, also exhibit an olfactory area in neopallial insula but the input is by way of the olfactory cortex. With fewer synapses between receptor and neocortex, one would expect the neocortical olfactory zone to respond to stimuli sooner than the adjacent cortex receiving taste stimuli. Such a temporal sequence may provide a means for olfactory stimuli to modulate taste responses at the cortical level.

Proposed Course: Thalamic relationships with the olfactory recipient zone of the neocortical insula will be sought using the HRP method.

Publications:

Switzer, R.C.: Wrinkle-free mounting of sections from gelatin embedded brains. Neuroscience Letters, 12: 103-105, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00875-02 LBEB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Identification of Forebrain Units that Discharge in Phase with Respiration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
	A.H. Orr	Research Psychologist	LBEB NIMH
	M. Eckardt	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)

William J. Vaughn, Research Services Branch, NIMH
Richard Radna, Dept. of Neurosurgery, Mt. Sinai Hospital, New York, New York

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present project is concerned with the question of interoceptive (visceral) influences on neuronal activity of the forebrain. In an earlier microelectrode study on awake, sitting squirrel monkeys, it was shown that vagal volleys reset the respiratory cycle and entrained units in certain structures of the forebrain to discharge in phase with respiration. A subsequent study in which the start of the expiratory phase was used to time the beginning of recordings revealed that about 10 percent of neurons in certain parts of the forebrain discharge in phase with respiration. In addition to obtaining more information about the distribution and incidence of such units, it was the purpose of the present study to learn whether or not their phasic discharge was related to inputs from the olfactory nerves. It was found that the respiratory-related phasic unit activity could be recorded following the destruction of the olfactory nerves.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In last year's work on this project it was found that somewhat more than 10 percent of units in certain structures of the forebrain discharge in association with respiration. In the present experiments we are continuing to explore the forebrain in order to obtain more complete information about the distribution, incidence, and firing patterns of such units. In addition, experiments are being conducted in order to learn whether or not such units can be found in animals deprived of olfactory innervation.

Methods Employed: The stereotaxic device employed for exploring the brain with microelectrodes in gently restrained, awake sitting squirrel monkeys has been described in preceding reports. For extracellular recording, glass micropipettes are used that have a DC resistance of 5 to 10 megohms. A small thermistor placed near the left nostril of the monkey, provides a means of obtaining a stable recording of the spirogram and triggering the oscilloscope. During an experiment both the spirogram and the unit discharge are displayed on an oscilloscope. Recordings of unit activity, the spirogram, the EKG, and other measures are permanently stored on magnetic tape which allows a retrospective computer-assisted analysis of the data. The recording system also provides on-line analysis. Three methods are used for showing a relationship between unit discharge and respiration--namely, a computer write-out of (1) an expectation-density histogram; (2) a normalized expectation-density histogram; and (3) a time series correlation.

In experiments requiring absence of olfactory input, the olfactory nerves are surgically sectioned.

Major Findings: To date 932 units have been studied while exploring 47 tracks in five animals (#409, #237, #410, #416, #333). Units discharging in association with respiration have been found in the hippocampus, amygdala, claustrocortex, putamen, tail of caudate, lateral geniculate body, supracingulate cortex, cortex deep in the sylvian sulcus, and dorsolateral cortex.

In the most recently explored animal (#333), the olfactory tracts were shown to be completely sectioned. Units discharging with respiration were found in CA4 and the prosubiculum of the hippocampus, the lateral nucleus of the amygdala, sylvian cortex, and dorsolateral cortex.

The results suggest that during exploration with a microelectrode passing through the forebrain, one may expect that about 10 to 15 percent of the isolated units will discharge in association with respiration.

Significance to Biomedical Research and the Program of the Institute: There is evidence derived from the study of psychomotor epilepsy that a sense of personal identity and the capacity for memory depend on an integration of externally and internally derived experience. The mechanisms by which visceral systems influence functions of the forebrain have remained a perplexing question. The present study suggests that respiratory activation of neurons of the forebrain may help maintain a central excitatory state and thereby promote a state of wakefulness and attention, as well as affect other psychological processes known to be modified by respiratory changes. The present experiments indicate that the discharge of cerebral neurons in association with respiration does not depend on an input from the olfactory system. A next step in this research is to investigate the effects of sleep on the "respiratory" neurons.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00834-03 LBEB

PERIOD COVERED
July 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Social Group Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. L. Hill	Visiting Associate	LBEB NIMH
Other:	J. H. Burkholder	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)
none

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: .3	PROFESSIONAL: .2	OTHER: .1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Examination is being made of the social/spatial organization of freely growing, confined populations of house mice. Mathematical and statistical techniques are utilized to analyze group structure in time and space. Particular emphasis is placed upon determining those factors that are related to social group development during the early phases of population growth. It is essential to know if early changes in the characteristics of social/spatial organization are related to the cessation of reproduction and eventual decline to extinction of high density populations. For example, in one population the social organization collapsed from a somewhat territorially organized society consisting of several individually identifiable groups into a single, homogeneously distributed group: there was a reduction in reproduction following the social collapse that may have led to the eventual decline and extinction of the population.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the principles of group organization, structure, and intragroup communication that operate to foster development and maintenance of optimum-sized groups, within (i) growing, (ii) stable, (iii) overcrowded, and (iv) declining populations. Social roles of individuals within groups, as well as the contribution of group stability to population survival, are being investigated.

Methods Employed: Cluster analytic techniques, including varimax rotation, were used to develop factor-loadings or weightings of association scores in the large matrices analysed. The data analyzed by these methods were the inter-animal distances between diurnal resting site locations by all animals in three freely-growing laboratory populations of house mice during each of fourteen censuses conducted during the early phase of population growth. Animals were assigned to groups on the basis of their degree of association with the mathematical factors identified by these procedures. Various statistical methods are being used to determine stability of these groups in time and space. (Because of the large amount of data and complexity of the analytic method, we estimate that over 300 hours of central processing time on the LBEB's PDP 11/40 computer had been utilized in our original analysis. Use of this computer has been of great value to our continuing evaluation and interpretation of the data.)

Major Findings: Our preliminary analysis indicated that the dynamic, constantly changing social/spatial organization within increasing populations became stable as population density became high. In the largest, fastest growing population there was a coalesence of several social groups into what we had originally thought were four large groups. On further inspection, we have found that the population was evenly distributed within a square enclosure (approximately the same number of animals nested along each of the 4 walls) and the mathematically determined center of each group was near its unique corner. In a square enclosure within which a population is relatively evenly distributed, the only unique sets of interanimal distances are those between animals on adjacent sides of each corner, and it was this phenomenon which caused our clustering technique to recognize four groups instead of one. Therefore, we now conclude that the population did not coalesce into four large groups but into one very large group. This total collapse in the spatial/social organization may have precipitated the eventual cessation of reproduction that led to the extinction of the population.

Proposed Course: To be discontinued.

Significance to Biomedical Research and the Program of the Institute: All animals, including human beings are adapted to develop and live most effectively within small, stable, social groups. However, urban induced anonymity, frequent change of residence, and crowding reduce the probability that meaningful interactions will occur at the appropriate intensity and frequency. Our studies of the principles of group organization among rodents contribute to the understanding of the requirements of group living.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00835-03 LBEB

PERIOD COVERED
July 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Crowding and Social Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J. L. Hill	Visiting Associate	LBEB NIMH
Other:	J. B. Calhoun	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH
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Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.0	OTHER: 2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with phenomena characterizing a house mouse population as it proceeds through successive stages of crowding in an octagonal, designed habitat. The 8 pie-shaped cells of the habitat are subdivided into 2 subcells with the nest boxes in the counter-clockwise subcell about one-half the distance from the resources as are those in the clockwise subcell. The growth of the population has been controlled, such that after the adult population reached 100, the population has been allowed to double every 210 days and now stands at 1600 adults. With increasing density there have been several changes in social structure and in space utilization. At lower densities more animals nested in the counter-clockwise subcells than in the clockwise subcells, while at high density there are about equal numbers of mice nesting in each subcell. Within the habitat, in 4 adjacent cells there are (i) more adults, (ii) many more adults nesting in public space, and (iii) higher infant mortality than in the other 4 adjacent cells. These differences are suggestive of a developing behavioral sink in that half of the habitat with the higher density.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine how (i) the physical structure of the environment and (ii) the behavioral and social history of members of the population influence composition and stability of social groups.

Methods Employed: All procedures of handling the mice and making observations follow a 210 day duration recurrent cycle. During the first 140 days of the cycle all progeny born are removed before they in turn produce progeny. This tactic provides adults with experience in rearing young. Post-reproductive adults are then removed from the population. The final 60 days of each 210 day cycle is devoted to establishing the next generation. Enough young are permitted to survive to double the entire population from the number of adults present before removal of the post-reproductive individuals. By this strategy, each successive generation is produced by experienced prime reproductive aged survivors of the two prior generations. Identification of each mouse is accomplished prior to weaning by the attachment of a pair of numbered metal ear tags. In a predetermined census schedule all mice are captured during the least active phase of their daily activity cycle when these nocturnal animals are associated with their preferred diurnal resting residences. Analysis of this resting residence data is restricted to the last of the sixth generation surveys: all young born into the population from this time are members of the seventh generation. Other data collected are related to the distribution in the habitat of the total population, adult population, juvenile population, and infant mortality.

Major Findings: Many changes in the spatial distribution of the population have occurred during the most recent doubling of the population size from 800 to 1600 mice. At the lower density more animals nested in the nest boxes in the counter-clockwise subcell than in the clockwise subcell; at the present density there are about equal numbers of mice nesting in the nest boxes of both subcells. Adults that nest in public space, on platforms between wire tunnels on the walls of the enclosure or on the floor, show a preference for the clockwise subcells.

Young adult mice are approximately evenly distributed among the 8 cells of the enclosure. Sixty-two per cent of the old adults prefer the four contiguous cells 6, 7, 8, and 1. Of the 61 per cent of all old adults that nested in public space, 64 per cent were found in the above four cells. Infant survival in cells 6, 7, 8, and 1 was only about 44 per cent while 58 per cent of the infants survived in the other four cells. The clustering of old, non-reproductive individuals and poor maternal care by reproductive individuals are indicative of a developing "behavioral sink" in cells 6, 7, 8, and 1.

With the beginning of the production of the 7th generation, we have observed the following changes in behavior: 1) in males, territorial behavior is being replaced by abnormal nipping behavior; 2) there has been a marked increase in aggression by females; 3) young males spend much of their active time forming and reforming into clusters of animals sitting side-by-side, all facing in the same direction on the low partitions that subdivide the enclosure; and 4) there appears to be a marked increase in mortality of very young infants.

Proposed Course: To be continued.

Significance to Biomedical Research and the Program of the Institute: This demonstration that design of the physical environment can enhance group stability suggests that further investigation on the effects of experimental manipulation of the physical environment on animals may contribute to development of architectural design principles that may ameliorate social stress on the human level. The new principles of design we introduced in the current environment were more effective than we had anticipated. They sufficed to push the optimum density towards double or possibly more than the anticipated 100 adult mice, perhaps by creating more clearly demarcated subunits of the physical area within which individuals could associate.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00836-03 LBEB

PERIOD COVERED
July 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Conceptual Adaptation and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	R. W. Ridel	Research Psychologist	LBEB NIMH
	G. A. Bagley	Computer Programmer	LBEB NIMH
	H. E. Cascio	Electronics Engineer	DRS BEIB NIH
	D. R. Ahuja	Visting Fellow	LBEB NIMH
	G. G. Ross	Inter-Governmental Exchange Fellow	LBEB NIMH
	R. A. Newell	Electronics Engineer	STD NIMH
	L. E. Olson	Physicist	TEKTRONICS, INC.

COOPERATING UNITS (if any)

BEIB, NIH
STD, NIMH

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.2	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By utilizing a hybrid strain of rats, Rattus norvegicus, a paradigm of human culture is being explored. This paradigm is based on the following theoretical considerations: The evolution of species of mammals, including human beings, has resulted in optimum group sizes of 12 adults. Since each group occupies a fixed territory, eventual occupation of all available space by such groups culminates in a stable adult population of the species. However, if conditions permit, an elaboration of social roles (particularly collaborative ones) and development of a more complex communication network, then more individuals can survive in a given area without a stressful increase in rate of interaction among members of the population that would otherwise trigger limitation of population size. In our experimental study 24 rats were introduced into each of two bilaterally symmetrical, compartmentalized habitats. After initial and equal distribution of animals throughout the habitat by number and sex, restructured social organizations became evident in both experimental and control populations, with the alpha male and half the females in both occupying one side of the habitat, and all remaining animals inhabiting the other side.

Project Description:

Objectives: The portion of this several-year-duration project that was conducted this year has the objective of establishing the character of the social structures in the experimental and control population prior to introduction of cooperative-role contingencies to be made operative by the computer-guided data acquisition system in the experimental population.

Methods Employed: Ss are Norway rats representing a double F1 cross of 2 domesticated and 2 wild strains. Population surveys are conducted every 21 days, with capture location, growth, health of the animals, and the condition of nests being recorded on each occasion. Young are removed, and litter condition, age, and capture location recorded during each survey period. Pelages of all animals are continually marked to assist in visual recognition during regularly conducted staff observations. Movement patterns of animals are constantly recorded by the computerized detector system. (The efficiency of this system has been dramatically improved through the continued collaborative efforts of URBS and BEIB staff.) As a prelude to this main study, a pilot one was conducted in a simpler compartmentalized habitat with 16 rats. Computer analyses are being conducted to determine the effect of required cooperation to obtain water on joint compartment occupancy.

Major Findings: Results cover the 133 days after colonization. Each of the two habitats is divided into two identical sides except for wall color; one side has a light tone, the other a dark tone. The dark side of each habitat was preferred by these nocturnal animals as indicated by residence of the alpha male and his harem of 6 females. These animals (high status group members) and the remaining 11 males and 6 females (low status group members) which inhabited the light side, maintained consistent residence in their respective habitat sides throughout this portion of the study that lacked demand for cooperative behavior. High and low status group members were differentiated by movement (social velocity) and reproduction. Low status group members were less active than members of the high status group. Excellent reproductive success (80 litters survived out of 86 recorded pregnancies) indicates that the design of the habitat is relatively optimum for a population of 24 rats. Despite an equal number of females in both high and low status groups, nearly 70% of the litters captured were found within the territorial confines of the high status group - suggesting that harem members of the alpha male conceived more frequently than did females in the low status group and/or that females in the high status group more frequently carried their pregnancies to full term. Between-group differences with respect to movement and reproduction suggests social structure influences behavioral expression. The hybrid rats of these two populations, that are housed in extremely complex environments, have developed more complex behaviors and express an enhanced adaptive capacity in comparison with more genetically uniform strains of wild and domesticated rats of eleven populations previously studied in simpler habitats. Examples include (1) construction of more complex nests, (2) circumvention of investigator introduced barriers, and (3) evincing less fear of investigators by remaining out of retreat spaces and obviously calmly watching the investigators who enter their habitat.

Proposed Course: To be continued.

Significance to Biomedical Research and the Program of the Institute: Our theory of the elaboration and persistence of human culture, which we are investigating by a paradigm that utilizes rats as subjects, proposes that hereditary diversity and environmental complexity are necessary conditions. The remarkable enhancement of learning and adjustment capacity exhibited by the rats in our study provide confirmation of the important function played by genetic diversity and environmental complexity. To the extent that capacity to learn and adjust represent desirable traits conducive to better mental health it may be noted that scientists and technologists currently appear to be emphasizing just the opposite. It is fairly dangerous now for a scientist's professional status to espouse that heredity plays a role in social life, and technologists (as well as scientists by default) are promoting increasing uniformity of the built environment. Our studies suggest the need for more inquiry about these two categories of variables.

Publications:

Calhoun, J. B.: Biological basis of the family. In Sagar, R. R. (ed.) 1978 Georgetown Family Symposia, Vol. III (1975-1976), A Collection of Selected Papers. Washington, D.C.: Georgetown University Family Center. Pp. 52-67. 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00837-03 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Alterations of Vocalization Induced by Crowding and Forced Cooperation in Rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J. L. Hill	Visiting Associate	LBEB NIMH
Other:	J. H. Burkholder	Guest Worker	LBEB NIMH
	J. B. Calhoun	Research Psychologist	LBEB NIMH
	W. J. Vaughn	Systems Programmer	STD NIMH

COOPERATING UNITS (if any)

STD, NIMH

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.6	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

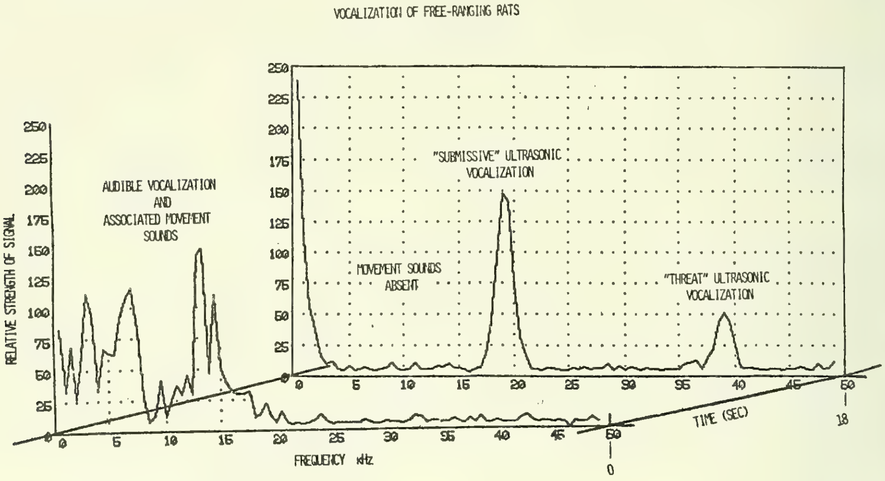
This project is designed to determine if the frequency and character of rat vocalizations change as a function of social experience. Two populations of rats will be compared: One population will be trained to engage in cooperative behavior patterns to satisfy food and water requirements. The other population will serve as an experimental control group and will not be required to perform cooperative behaviors. It is expected that as each population triples in size those rats required to perform cooperative behaviors will exhibit a larger repertoire of vocal patterns than will the control group, while crowded control rats will exhibit vocalizations of diminished complexity. Preliminary data indicate that rats utilize audible (to humans) and ultrasonic vocalizations in social and sexual interactions. These data represent baselines of normal vocalizations collected prior to either inducement of cooperative behavior in the experimental population or increase in population of both groups.

Project Description:

Objectives: To determine if the quality, quantity and pattern of rat vocalizations change when the animals are required to perform complex cooperative behaviors to meet their physiological needs.

Methods Employed: Vocalizations are detected by a B&K condenser microphone (Dynamic range = 20Hz-200kHz) equipped with a parabolic reflector, and classified according to type and frequency by the URBS PDP 11/04 computer and Tektronix 5L4N Spectrum Analyzer. Simultaneous recording of vocalizations and direct observations of rat activity are being made to determine the types of vocalizations that are associated with specific behaviors.

Major Findings:



We present an example of the baseline vocalization data we are collecting for comparison with that to be collected when experimental treatments are applied to two populations of Norway rats. Three types of rat vocalization are depicted in the figure above: 1) an audible vocalization accompanied by sounds made by one rat as it runs away from an attacker; 2) a 19 kHz "submissive" vocalization uttered by a subordinate rat in an attempt to prevent its being attacked by a nearby dominant individual; and 3) a 39 kHz "threat" vocalization uttered by a dominant individual. This figure represents two approximately 1/8 second "slices" of time, the first during an attack and chase of one rat by another, and the second, 18 seconds later, while these two rats were confronting each other and were not moving. These vocalizations represent only a small sample of the wide variation in vocalization wave length and type of social interaction within which rats vocalize. For example, male rats utter a post-ejaculatory "mating song" which is very similar to the 19 kHz "submissive" vocalization in the figure.

Proposed Course: To be continued.

Significance to Biomedical Research and the Program of the Institute: In humans, stressful social interactions produce short term changes in vocal communication. However, it is not known if long term stress produces long-term changes in vocal communication. In the present project we are using an animal model to determine if long-term stress induced by crowding results in changes in the vocal communication of rats, and if the effects of the stress may be ameliorated in rats that are induced to perform cooperative behaviors.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00838-02 LBEB

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Theoretical Population Dynamics; An Evolutionary World Population Model.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	D. R. Ahuja	Visiting Fellow	LBEB NIMH
	G. G. Ross	Intergovernmental Exchange Fellow	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A model of past and future changes in the human world population has been derived. It is partially based on a theory of the course of evolution that sees enhancement of awareness and responsiveness as the major central tendency. Preservation of this central tendency requires a gradual shift from an increasing to a decreasing human population. The change between these two trends has just begun. A maximum of nine billion people will be reached in 2065 after which decline in numbers ensues. By 1975 world-wide declines in fertility indicated that the greater than exponential rate of population increase of the past several millenia had ceased. Fertility, by nation, declines at 1.5% per year until replacement level is reached, after which its decline is much slower, reaching a minimum of 0.75 GRR in 2155. By 2175, when the world population will have declined to about 5.3 billion, a new evolutionary era of population change will have emerged in which each successive population halving will take four times as long as the prior halving. A model of intra-specific competition suggests that preponderance of non-reproducing older ages individuals by 2080 may inhibit expression of social roles sufficient to threaten human survival.

Project Description:

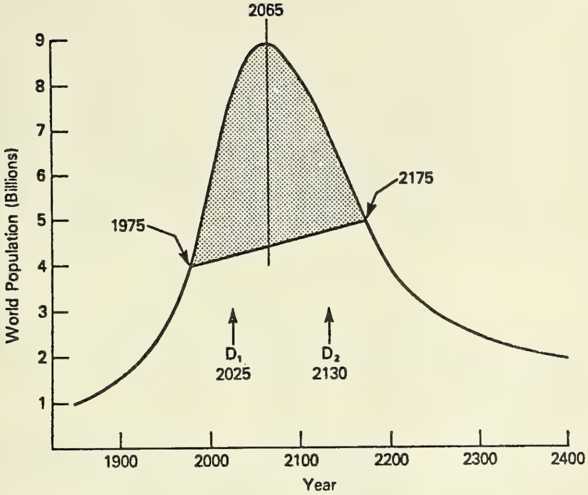
Objectives: To develop a theoretical world model of change in the size of the human population that will help guide the human species through the several-hundred year crisis period ahead and then to enter a new era of evolution that will permit the members of the human species to continue to increase their capacities for awareness and responsiveness. Specifically, we wish to determine the nature of the transition between an increasing and declining phase of change in human numbers.

Methods Employed: Two theoretical equations describe the change in world population over time. The von Foerster-Meyer equation describes changes in numbers to the present as following a course in which each successive doubling required only half the time as the prior doubling. The Calhoun-Ahuja equation, which should apply at some point in the future, predicts that each halving of world population will take four times as long as the prior halving. We established the character of the transition between these two equations by the following steps. Utilizing U.N. census data and analyses, we found that once fertility begins to decline, decrease continues at 1.5% per year until replacement level is reached, after which decrease in fertility is much slower. We then varied minimum fertility and date of minimum fertility, incorporating best present estimates of life expectancy, and by employing appropriate U.S. Census Bureau programs held at DCRT, we ran a series of simulations to optimize lowest level of maximum population consistent with the earliest date for minimum fertility and highest level of minimum fertility that would provide a smooth transition into the Calhoun-Ahuja equation without abrupt fluctuation in fertility. These simulations were carried out separately for more and less developed regions of the world and the results summated.

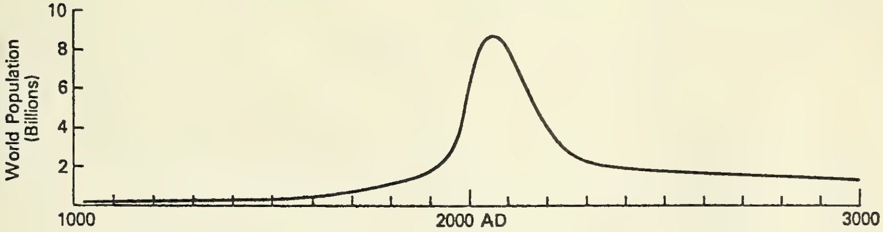
Major Findings: Figures 1 and 2 below depict the change in world population over time. The stippled area in Figure 1 denotes the transition between the two equations for population change. Termination in 1975 of full operation of the equation describing increasing population denotes the end of the 40 to 50 thousand year era of cultural evolution. Continued function of this equation after 1975 would have produced an infinite population by the D1 ("Doomsday") date of 2025. Similarly the declining population equation assumes starting at an infinite sized population at the D2 ("Dawnsday") date of 2130. Interaction between the forces producing changes in population size described by these two equations produces the 200 year transition period during which maximum population will likely be just below nine billion. From a longer time perspective (Figure 2) the 1975 to 2075 peak in world population may be seen as a short-duration phase between two eras of much lower rate of change in number. The maximum mean age of world population will be reached in 2080 with 58% of the population over 40 years of age. At that time only about 10% of the total population is anticipated to be involved in the production of children. Also at around 2080 the pre-reproductive segment of the population will vary around 17%.

Figures 1 and 2 next page.

WORLD POPULATION



EVOLUTIONARY POPULATION TRANSITION



Proposed Course: To be discontinued.

Significance to Biomedical Research and the Program of the Institute: The world has just entered a unique 200 year episode of change between two eras of evolution in which during the transition both the rate of change in many phenomena, and the change in character of phenomena will be greater than in any like duration period in history. To the extent that mental health is influenced by capacity to adapt to such change, this next 200 years will comprise a time of maximum threat to mental health. Prevention of mental illness during this time must take into account role changes associated with commensal relations with information processing prostheses. The marked reduction of pre-reproductive aged individuals shortly after the peak in world population will produce a threat to their assumption of effective adult social roles. According to the current theory of Ross, the world population may rapidly go to extinction at this time from failure to acquire adult roles.

Publications:

Calhoun, J. B., and D. R. Ahuja: Population and environment: an evolutionary perspective to development. In Hauser, P. M. (ed) World Population and Development: Challenges and Prospects. Published by Syracuse Univ. Press. 1979.

Ross, G. G.: Non-linear perturbations of difference equations in population dynamics. Technological Forecasting and Social Change. In press.

Ross, G. G., and N. A. Slade: A Predator-Prey Viewpoint of a Single Species Population. Journal of Theoretical Biology. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00843-01 LBEB

PERIOD COVERED
July 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Conceptual Prosthesis of the Brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	G. A. Bagley	Computer Programmer	LBEB NIMH
	M. D. Murtha	NIMH Contractor	ASMER
	P. E. Heinig	NIMH Contractor	ASMER

COOPERATING UNITS (if any)
Association for the Study of Man-Environment Relations under NIMH Contract #278-77-0055(SM) with J. B. Calhoun as Project Officer.

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been proceeding since 1966. Concept analysis of 600 articles and books led to the hypothesis that there are 1625 preverbal, experiential generic concepts functionally arranged as an extended icosahedral, 7-step, network. "Tradition", in the sense of genetic and learned rules guiding behavior, lies at the center. If memory traces of experience and thought are stored at the 1625 nodes, it should be possible to simulate brain functions utilizing condensed text excerpts, indexed for generic concepts, as memory units to be reassembled as new sequences that logically intersperse excerpts from many authors to form a manuscript resembling one produced by a single person. Recognizing that full simulation of such brain function is a few years off, two developmental steps have been pursued: (1) 162 authors provided highly condensed statements for the domain encompassing "adaptation, population, environment"; and these have been organized and analyzed relative to the developing theory of the generic concept network. (2) An exhaustive survey of the domain encompassing "the built environment and health" to determine the characteristics of an information access system capable of identifying the most important published writings to be subjected to excerpt condensation.

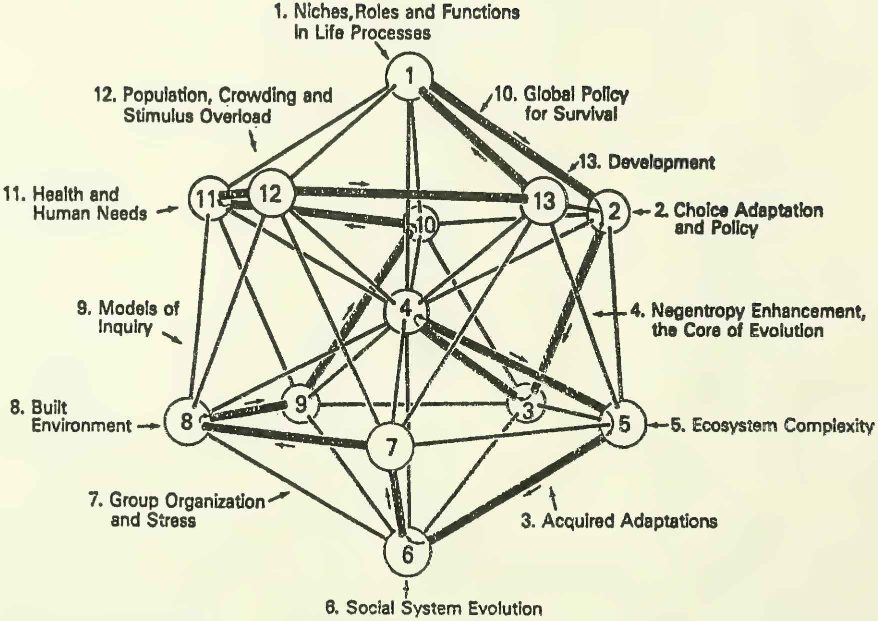

1. Niches, Roles and Functions in Life Processes
10. Global Policy for Survival
13. Development
2. Choice Adaptation and Policy
4. Negentropy Enhancement, the Core of Evolution
5. Ecosystem Complexity
3. Acquired Adaptations
6. Social System Evolution
7. Group Organization and Stress
8. Built Environment
9. Models of Inquiry
11. Health and Human Needs
12. Population, Crowding and Stimulus Overload
1
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Fig. 1. Center-point icosahedral network of chapters for the book, "Adaptation, Population, Environment--Design of Evolution."

Project Description:

Objectives: To develop bases for further elaboration of theory and empirically substantiated insights about how major concepts are functionally interrelated within the mammalian brain and to develop the methodology whereby computer stored excerpts from the literature may be reassembled into new thought sequences to represent a conceptual prosthesis to the brain.

Methods: Two conceptual domains have been explored. They are: A, "Built Environment and Health" and B, "Adaptation, Population, Environment." In the "A" domain a number of searches were conducted for relevant literature occurring in eight online citation data bases. In addition, an extensive survey was conducted with the aid of 200 consultants to determine the scope of the domain with particular reference to research idea generators, principle journals in which they publish, and the scope of objectives and concepts utilized. For the "B" domain an exhaustive search identified 450 researchers as potential contributors of condensed statements about "important researchable problems." 162 of these prepared the desired 1800-word statements. The statements were subjected to an indepth content analysis to force them into thirteen aggregates that most closely fit the centerpoint icosahedral model. Then for publication purposes the icosahedral network was unfolded to form the one linear sequence that maximizes concept and content similarity between adjacent 'chapters' (see Figure 1, heavy line between nodes).

Major Findings: For the domain "adaptation, population, environment," the conceptual content analysis of the 162 1800-word statements revealed that they fell fairly naturally into 13 clusters or chapters. Further analysis, including interstatement bonding by shared citations of the same reference authors, revealed that one arrangement out of 480 million possible ones provided the highest possible degree of network bonding among chapters. Furthermore, one route (shown in heavy lines in the above figure) over the network provided the highest sum of bonding between adjacent linearly arranged chapters.

For the domain of "built environment and health" it was found that relevant literature is spread over an extremely wide range of journals, and much important literature never becomes referenced in automated citation data bases. There is no existing institution capable of representing the wide range of research and applied professions to promote better access to such information.

Proposed Course: To be discontinued.

Significance to Biomedical Research and the Program of the Institute: The broad theory emanating from our research over the past 25 years at the NIMH holds that cultural evolution produced an extrapolation of biological brain function to form a socio-cultural brain formed of people linked to people in an ever larger communication network. This theory holds that, just as the biological brain reached maximum development 50,000 years ago, so it will be that very soon the sociocultural brain will also reach maximum development. Beyond that date, possibly passed already during the present decade, information processing prostheses--such as computer related systems--will begin to reach levels of sophistication sufficient to represent the initiation of a third category of brain evolution. Our work points to the opportunity of promoting the evolution of such prosthetic brains with the capabilities of interrelating ideas generated by many different persons in a fashion which essentially simulates how a single individual interrelates ideas within in a biological brain. This direction of inquiry culminates in a broad conclusion about brain research and mental health. Evolution is a process whereby the amount and diversity of information (negentropy) increase over time. Brain, as a generic concept, relates to the interrelationship of units capable of processing information effectively and increasing the diversity of information. Mental health increases to the extent that all individuals are involved in promoting the effectiveness of all three generic levels of brain functions. Therefore, progress in mental health can only come from brain research in this broad sense. Such research must develop theory coordinating the three level triune biological brain with the triune evolutionary stages of generic levels of brain evolution, the biological, the sociocultural, and the prosthetic.

Publications:

Murtha, D. M.: Feasibility Study on Improving Access to Information About the Impact of the Built Environment on the Health and Lives of People: Final Report. Association for the Study of Man-Environment Relations, 135 Pages. April 1979.

Murtha, D. M.: Feasibility Study on Improving Access to Information About the Impact of the Built Environment on the Health and Lives of People: Project References, Appendix I-V. Association for the Study of Man-Environment Relations. 200 Pages. April 1979.

Murtha, D. M.: Feasibility Study on Improving Access to Information About the Impact of the Built Environment on the Health and Lives of People: Project Consultants, Appendix VI-IX. The Association for the Study of Man-Environment Relations. 300 Pages. April 1979.

Murtha, D. M., and P. E. Heinig, Jr.: The Impact of the Built Environment on the Health and Lives of People: Selections From the Medline Database. Association for the Study of Man-Environment Relations, Inc. ASMER Information Access Project. 500 Pages. November 1978.

Heinig, P. E., Jr.: Online Information Retrieval Pertinent to Human Health and Well-Being in the Built Environment, An Exploration of Seven Online Files. Association for the Study of Man-Environment Relations. 200 Pages. December 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00881-23 LCM

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Intermediary Energy Metabolism in Mammalian Brain (new title) formerly called: The Mechanism of Action of Thyroxine and Its Relation to Cerebral Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. Kaufman	Research Chemist	LCM NIMH
OTHER:	T. Nelson	Staff Fellow	LCM NIMH
	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has taken a new direction which includes studies on the origin, mode of action, and metabolism of γ-hydroxybutyrate, (GHB), a compound which is a normal constituent of mammalian brain and which is of increasing pharmacological interest. When administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state and a profound depression of cerebral glucose utilization. This compound is being used clinically in Europe as an anesthetic adjuvant. Preliminary trials indicate that it is effective in the treatment of narcolepsy and may be of use in the treatment of stroke.

GHB, and an enzyme capable of metabolizing it, have been found in tissues such as liver and kidney as well as in brain. This enzyme, an $NADP^+$-linked alcohol oxido-reductase which interconverts GHB and succinic semialdehyde, has been purified from hamster liver and partially purified from hamster brain. Studies completed on this enzyme include substrate specificity, enzyme kinetics, molecular weight determination, and inhibition studies with the anticonvulsant agents, diphenylhydantoin, amobarbital, and valproate.

PHS-6040
(Rev. 10-76)

Project Description:

This project is directed at understanding the role of naturally-occurring γ-hydroxybutyrate as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions. In addition to the pharmacological effects already mentioned (flattening of the EEG, trance-like state and depression of cerebral glucose utilization) it has recently been reported that γ-hydroxybutyrate increases the survival of animals which have been made hypoxic. The authors report that the blood and brain tissues of animals which have received γ-hydroxybutyrate showed marked changes in the levels of some intermediates in carbohydrate metabolism as well as changes in levels of high energy phosphate compounds. These studies have led to the proposal that γ-hydroxybutyrate may be useful in the treatment of stroke. Because γ-hydroxybutyrate is a compound which is used clinically, and because it is a naturally-occurring compound, we feel that it is important to understand not only pathways of metabolism of γ-hydroxybutyrate, but also the factors which control these pathways.

A study on the purification and characterization of an $NADP^+$-linked alcohol oxido-reductase capable of interconverting γ-hydroxybutyrate and succinic semialdehyde has been completed and published. This enzyme has been purified 300-fold and found to exhibit a single band on gel electrophoresis. The protein is a monomer with a molecular weight of ∿ 31,000. The enzyme is inhibited by two anticonvulsant agents, amobarbital and diphenylhydantoin, as well as by valproate, a drug used in the treatment of epilepsy. Pyrazole, the specific inhibitor of alcohol dehydrogenase (NAD^+-linked) does not inhibit this enzyme. Diethyldithiocarbamate, the reduced form of the drug antabuse, as well as KCN, both metal-chelating agents, inhibit the enzyme. This suggests that the enzyme may contain either copper or zinc as an integral part of the protein. This possibility is currently being investigated.

In vitro, alcohol dehydrogenase (NAD^+-linked) (ADH) is also capable of catalyzing the interconversion of γ-hydroxybutyrate and succinic semialdehyde (SSA). The Km for γ-hydroxybutyrate with alcohol dehydrogenase is, however, an order of magnitude higher than the Km with the $NADP^+$-linked oxido-reductase. In vivo studies on the half-life of $[^{14}C]$γ-hydroxybutyrate (in plasma) in the presence and absence of various inhibitors have been carried out in an effort to assess the contribution of these two enzymes to the metabolism of γ-hydroxybutyrate in the whole animal. Results of these studies show that pyrazole, a potent inhibitor of ADH, does not affect the half-life of γ-hydroxybutyrate. The same concentration of pyrazole produced severe inhibition of ethanol metabolism in the whole animal. This suggests that ADH is probably not the enzyme responsible for the oxidation of γ-hydroxybutyrate in the whole animal. Preliminary results show that two compounds which inhibit the oxidation of γ-hydroxybutyrate catalyzed by the $NADP^+$-linked oxido-reductase, dilantin and L-gulonate, increase the half-life of γ-hydroxybutyrate in the plasma (indicating a decreased rate of metabolism). These results lend support to a role for the $NADP^+$-linked alcohol oxido-reductase in the metabolism of γ-hydroxybutyrate in the whole animal.

Changes in the levels of various intermediates in carbohydrate metabolism, most notably lactate, as well as changes in high energy phosphate compounds following administration of γ-hydroxybutyrate, have been reported. We are currently investigating the biochemical basis for the changes which have been observed both in normoxic and in hypoxic animals.

Identification of γ-hydroxybutyrate in tissues other than brain is a project which is nearing completion. It has been found that both liver and kidney contain higher levels than those observed in brain tissue. The compound has been detected in a number of other tissues, although the levels are not as high as those found in kidney and liver.

The behavioral effects of exogenously administered γ-hydroxybutyrate are very similar to those seen in hibernation, a phenomenon that is still not understood. The possibility that γ-hydroxybutyrate may be involved in hibernation is being examined in this laboratory. Preliminary studies in the hibernating hamster indicate that there is a significant increase in the level of the enzyme that oxidizes γ-hydroxybutyrate in brain but not in liver. These studies will be continued and will be extended to the measurement of γ-hydroxybutyrate levels in brain in hibernation. Progress in this area will be greatly facilitated by the recent acquisition of a gas chromatographic unit with a nitrogen-detector, which should simplify the assay of γ-hydroxybutyrate.

A new spectrophotometric assay for the detection of γ-hydroxybutyrate has been devised. It is based on the use of the purified $NADP^+$-linked alcohol oxido-reductase and the synthetic co-factor, 3-acetylpyridine-adenine dinucleotide phosphate. The substitution of the synthetic co-factor shifts the equilibrium of the reaction $GHB + NADP^+ \rightleftharpoons SSA + NADPH + H^+$ far to the right so that the GHB may be estimated by the amount of reduced co-factor which has been produced. We anticipate that this assay will prove useful in the estimation of blood and tissue levels of γ-hydroxybutyrate.

This project will have a continued interest in ketone body metabolism in normal as well as in pathological states. D-β-Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D-β-hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from catabolism, and it is converted to D-β-hydroxybutyrate by the action of the enzyme. Once formed, however, D-β-hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role the enzyme plays. Why does the body not utilize the directly-formed acetoacetate rather than convert it first to D-β-hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility existed that the role of the enzyme is protective to keep the acetoacetate concentrations low by storing it, in a sense, as D-β-hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their prior conversion to acetylCoA. The pathway is the conversion of β-hydroxybutyrate to acetoacetate which then displaces succinate from succinylCoA to form acetoacetylCoA. The acetoacetylCoA is then converted to acetylCoA and enters the Krebs Cycle. For every acetoacetate molecule thus metabolized, there is one succinylCoA degraded. SuccinylCoA is required for the formation of GTP which is the precursor of cyclic GMP. Excessive use of ketone bodies can theoretically, therefore, lead to depletion of available GTP and, therefore, also cyclic GMP. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain. These studies will be more aggressively pursued during the next year.

This project will continue on the course which has been outlined. It will include: 1) further kinetic studies with $NADP^+$-linked alcohol oxido-reductase; 2) determination of the presence or absence of a metal in this enzyme; 3) _in vivo_ studies to determine the effect of various drugs, normal metabolites and alterations in physiological state on the metabolism of GHB; 4) continued investigation of GHB metabolism in the hibernating animal; 5) investigation of the interaction of GHB with other pathways of carbohydrate metabolism; 6) study of the biosynthetic pathway for GHB; 7) developmental studies on the levels of GHB and of the $NADP^+$-linked alcohol oxido-reductase in brain and other tissues. 8) This project will have a continued interest in ketosis, β-hydroxybutyrate metabolism and the relationship between ketosis and cyclic GMP levels in brain.

Publications:

1. Kaufman, E. E., Nelson, T., Goochee, C., and Sokoloff, L.: Purification and characterization of an $NADP^+$-linked alcohol oxido-reductase which catalyzes the interconversion of γ-hydroxybutyrate and succinic semialdehyde. _J. Neurochem._ 32:699-712, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00882-12 LCM

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on Regional Cerebral Circulation and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPLE INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT:

PI:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
OTHER:	D. Ingvar	Visiting Scientist	LCM NIMH
	C. Kennedy	Guest Worker	LCM NIMH
	M. Shinohara	Visiting Associate	LCM NIMH
	M. Miyaoka	Visiting Associate	LCM NIMH
	C. S. Patlak	Chief, Theoretical Statistics & Math.Br.	B NIMH
	K. Pettigrew	Mathemat./Stat., Theoret. Stat. & Math. Br.	B NIMH
	H. E. Savaki	Visiting Fellow	LCM NIMH
	J. McCulloch	Guest Worker	LCM NIMH
	M. Kadekaro	Guest Worker	LCM NIMH
	S. Suda	Visiting Fellow	LCM NIMH
	C. Smith	Staff Fellow	LCM NIMH
	W. Kuschinsky	Guest Worker	LCM NIMH
	P. Yarowsky	Guest Worker (NIGMS Fellow)	LCM NIMH

COOPERATING UNITS: T.Duffy, Cornell U., NYC; M.Phelps & D.Kuhl, UCLA, Los Angeles; W.Bunney & A.Pert, Adult Psychiatry Br.,NIMH; R.Post, Adult Psychiatry Br., NIMH; M.Mishkin, Lab. of Neuropsychology, NIMH; J.C.Gillin, Lab. of Clin. Psychopharmacology, DSMR, NIMH, J.DiMicco, Lab. of Clinical Sci.,NIMH; S.Rapoport, Lab. of Neurobiology, NIA, NIH.

LAB/BRANCH

Laboratory of Cerebral Metabolism, DBBR, NIMH

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
15.5	8.75	6.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [^{14}C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [^{14}C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping functionally, and therefore metabolically, linked structures in the CNS, the method is being used to map the functional visual pathways in the monkey. It is also being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

Project Description:

Previous work in this Laboratory several years ago led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed how to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively.

All the theoretical and technical problems have been solved, and the method has been completely operative. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of $[^{14}C]$deoxyglucose is injected, and arterial plasma concentrations of $[^{14}C]$deoxyglucose and glucose are measured from the time of injection till 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue $[^{14}C]$deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The original operational equation was valid only under conditions in which the plasma glucose level remained constant throughout the experimental period. This requirement was cumbersome because many experimental conditions cause the plasma glucose level to vary. A new operational equation was derived which does not have this restriction, but it requires knowledge of the half-life of the free glucose pool in the tissue. A method was, therefore, developed which measures the half-life of the free glucose pool for the first time. The method has been used in the rat, and the half-life has been found to average 1.2 min in the conscious state, 1.8 minutes in the anesthetized state, and to vary in proportion to the plasma glucose level. Aside from the usefulness of this method as an independent research tool, it has now made it possible to employ the $[^{14}C]$deoxyglucose technique in conditions in which the plasma glucose concentration varies during the procedure.

The method has been applied to normal conscious and anesthetized rats and conscious monkeys. The values obtained cannot be directly compared to any other data in the literature since measurement of local cerebral glucose consumption has never previously been possible, but the average of the local values is very close to the reported values for overall average brain glucose utilization in those two species. Anesthesia has been found to depress cerebral glucose consumption throughout the brain. Carbon dioxide inhalation has been shown to depress local glucose consumption even though it increases cerebral blood flow. Local cerebral blood flow has been found in the normal animal to correlate very closely with local cerebral energy metabolism, a relationship which had been hypothesized but never previously proved. Increased local functional activity produced by electrical stimulation or by experimentally induced focal seizures following local penicillin injection results in markedly increased local glucose consumption in appropriate regions of the CNS. The studies with experimental focal epilepsy have established the existence of a "mirror focus" contralateral to the epileptogenic focus. These results have established once and for all the often debated but never previously proved fact that energy metabolism in the CNS is closely coupled to functional activity as it is in all other tissues.

Studies have been completed in the rat on the effects of γ-butyrolactone, a drug alleged to block specifically dopaminergic synapses in the brain. A surprising result was that this agent, which produces a trance-like state, also depresses local glucose consumption throughout the brain. Indeed, the magnitude of the reduction, which is dose-dependent, can be as great as that seen in profound hypothermia, which is used by cardiac and neurosurgeons to reduce energy metabolism in the brain and thus protect it during surgical procedures necessitating interruption of cerebral blood flow. The effects of the drug are completely reversible. Normal consciousness, EEG, and behavior are recovered approximately 4 hours after the injection, and the animal appears normal. This finding may have enormous practical potentialities. It is conceivable that the beneficial effects of hypothermia without its potentially dangerous side-effects and complex technical procedures may be achieved with this drug. Studies are continuing to ascertain whether, in fact, it is safe to try it out in man for surgical procedures.

Studies have been completed on the effects of LSD and of acute morphine administration, morphine addiction, and morphine withdrawal on local cerebral glucose utilization. The morphine studies were carried out in collaboration with Dr. Werner Klee. These studies have demonstrated selective, dose-dependent depressions of energy metabolism in specific regions of the brain. Morphine addiction has been found to result in significant reductions of glucose utilization in a number of structures of the brain, particularly those of the limbic system. Acute withdrawal from addiction by naloxone administration results in a reversal of this reduction in several of those structures, most prominently in the habenula. An interesting observation is that naloxone alone, when administered to normal rats, reduces glucose utilization in many of the same structures affected by morphine addiction. Naloxone, which is used as a morphine antagonist, has generally been believed to be inactive by itself. These results suggest, however, that it is active, possibly by acting on the receptor sites normally acted upon by the recently discovered endogenous "opiate polypeptides", i.e., the enkephalins.

Studies have been completed on the effects of amphetamine in the rat. This dopaminergic agonist produces pronounced increases in glucose utilization in selected structures of the brain, in particular the components of the extra-pyramidal dopaminergic motor system, i.e., caudate nucleus zona compacta and zona reticulata of the substantia nigra, etc. A markedly affected structure was the sub-thalamic nucleus, which had previously not been suspected to be dopaminergic, but in follow-up studies based on those of this laboratory, has been found to contain an active dopamine-sensitive adenylcyclase. Similar studies are in progress on the effects of another type of dopamine-agonist, apomorphine, which appears to have similar effects on local cerebral glucose utilization. Studies are also currently in progress in collaboration with Drs. Agu Pert and William Bunney of the Adult Psychiatry Branch, NIMH, on the supersensitization of dopaminergic synapses by chronic administration of dopamine-blocking drugs. The results thus far clearly demonstrate metabolic correlates of the super-sensitivity phenomenon.

Studies on the effects of the β-adrenergic blocking agent, propranolol and the α-adrenergic blocking drug, phentolamine, have led to surprising results. These drugs have directly opposite effects selectively on the auditory system of the rat. Propranolol, which is presently being widely touted as an anti-hallucinogenic agent in schizophrenia and other psychoses, was found to depress markedly the rates of glucose utilization throughout the entire auditory system from the cochlear nucleus to the auditory cortex. In contrast, phentolamine, which has mild depressant effects in most other parts of the brain, has exactly the opposite effects in the components of the auditory system, namely a pronounced increase in glucose utilization. Studies have been completed extending the investigations to other adrenergic agonistic and antagonistic drugs. The acquired data is currently under analysis.

In the course of the development of the method it became apparent that even without complete quantification, the autoradiographs represented pictorial displays of the relative rates of energy consumption in all parts of the brain. For example, they demonstrated clearly that the synapse-rich areas, e.g., layer 4 of the neuropile in the cerebral cortex, are the most metabolically active areas of the cortex. It also became apparent that inasmuch as local glucose consumption changes in response to changes in functional activity, regions of the brain which were turned on or off functionally and metabolically in altered physiological states could be mapped simultaneously throughout the brain. The efficacy of this technique of mapping by "evoked metabolic response" has been demonstrated in the visual and auditory systems of the rat and monkey. All the regions involved in visual functions have, for example, been shown to alter activity and be displayed on the autoradiographs after occlusion of vision of one eye and stimulation of the other. By this technique the visual cortical locus of the representation of the blind spots of the visual fields has been demonstrated in the monkey and its shape and dimensions defined. The resolution of the method is surprisingly good. It has clearly demonstrated the ocular dominance columns described by Hubel and Wiesel, which are believed to represent the structural and functional organization of the visual cortex responsible for the integration of the two images from the two eyes in animals with binocular vision. Application of the method to the newborn monkey has demonstrated that these columns are already organized at birth. Patching of one eye in the newborn for 3 months leads to a reorganization of the visual cortex so that the portions representing the blocked eye are taken over by the portions of the visual cortex subserving the open eye. Such plasticity is not present in the mature monkey brain.

The method has also been used to study the visual systems of the albino and pigmented rats. It has shown that the visual system of the albino rat is more sensitive than that of the pigmented rat at very low light levels, but is overwhelmed or "fatigued" at higher light levels which cause a reduction in local glucose utilization in the visual structures. These studies have also demonstrated a linear relationship between the rate of local glucose utilization in central visual structures and the log of the intensity of retinal stimulation, indicating that local metabolic rate in the nervous system, like behavioral manifestations, obeys the Weber-Fechner Law.

Studies are in progress to determine the mechanisms by which glucose utilization is coupled to functional activity. One possibility is that increased functional activity increases extracellular K^+, and it is the increased extracellular K^+ that stimulates energy metabolism by activation of the Na^+,K^+-ATPase. Experiments have been carried out in which KCl is applied to the parietal cortex trans-durally by its topical application through a hole in the skull or directly on the pial surface of the cortex. Such application of the KCl markedly stimulates glucose consumption in the underlying cortex, probably by direct action, while depressing metabolic activity in subcortical structures, probably by reflex or neurogenic reduction of functional activity. This treatment is known to cause the phenomenon of spreading cortical depression during which the DC potential of the cortex is shifted negatively. Studies during the recovery from spreading depression (i.e., immediately following the return of the DC potential to normal) have demonstrated that there is still increased cortical glucose utilization, but it is distributed in a columnar pattern traversing the thickness of the cortex. These results give additional support to the accumulating evidence of the columnar organization of functional activity in the cerebral cortex.

The method has also been used to study the acute and delayed effects of hyper-osmolar opening of the blood-brain barrier. The barrier was opened by unilateral carotid arterial injections of hyper-osmolar mannitol. Shortly after the barrier is opened, there is metabolic evidence of punctate regions of seizure-like activity on the side of the barrier opening. The effect is apparently reversible, for those areas of markedly increased metabolic activity return to normal after a period of an hour or more following opening of the barrier.

The regional localization obtained with the [^{14}C]deoxyglucose method is achieved by the use of quantitative autoradiography. The autoradiographs provide pictorial representations of the relative, not the actual, rates of glucose utilization in all structures of the brain. They are ordinarily subjected to manual densitometric analysis from which local ^{14}C concentrations are derived and used in the operational equation to compute the actual rates of local glucose utilization. We have recently developed a computerized image-processing system to analyze and transform the autoradiographs into color-coded pictorial maps of the actual rates of glucose utilization throughout the entire CNS. The autoradiographs are scanned automatically by a computer-controlled scanning microdensitometer which measures the optical density of each spot, 25-100 μm, in the autoradiograph. These optical densities are stored in a computer, converted to tissue ^{14}C concentrations on the basis of the optical densities of calibrated ^{14}C plastic standards, and then converted by the computer to actual local rates of glucose utilization by solution of the operational equation. Colors are assigned to narrow ranges of the rates of glucose utilization, and the autoradiographs are then displayed on a color monitor in color along with a calibrated color scale for identifying the rate of glucose utilization in each spot of the autoradiograph from its color. These pictures are, therefore, complete color maps of the actual rates of local glucose utilization precisely localized in each 25-100 μm region of the CNS.

Recent technological developments with computerized axial tomography have made it possible to scan the human brain for local tissue concentrations of positron-emitting isotopes. Experiments have, therefore, been carried out with [^{18}F]fluoro-deoxyglucose, a positron-emitting analogue of [^{14}C]deoxyglucose, and the results demonstrate that it can be used in place of [^{14}C]-deoxyglucose as the tracer for the deoxyglucose method. This now makes it possible to use the method in man. In collaboration with Dr. M. Reivich of the U. of Pa., and M. Phelps and D. Kuhl of UCLA, the method has been adapted for human use. The NINCDS has ordered a scanner which should be operational soon. This instrument will be available for our use, and we are preparing protocols to apply it to studies of psychotic states, aging, and dementia in man.

The [^{14}C]deoxyglucose method measures glucose utilization which correlates with and appears to be a marker for local cerebral functional activity. There are, however, other parameters of cerebral function and structure of interest. We are, therefore, developing a comparable method for the quantitative determination of local cerebral protein synthesis. It is expected that this method should allow more pertinent and probing studies of the development of brain structure and function in normal and abnormal states. The model is similar to that of the glucose method, and progress is being made to bring the method to an operational state.

Several lines of further investigation are planned in this project. One line is methodological by which it is hoped to improve the resolution of the method to the microscopic or single cell level. Another line is the exploitation of the [^{14}C]deoxyglucose method at its present state in studies of the effects of aging, psychoactive drugs, metabolic comas, development and maturation of the brain, and hormones such as thyroxine known to have important actions on the maturation of the brain. Finally, the local protein synthesis method should be soon developed to the operational level and be ready for use in studies of brain development.

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13. Shinohara, M., Miyaoka, M., Sakurada, O., Sokoloff, L., Jarvis, C. D., and Mishkin, M.: Mapping the primate visual system with 2-[^{14}C]deoxyglucose. In Gotoh, F., Nagai, H., and Tazaki, Y. (Eds.): *Cerebral Blood Flow and Metabolism*. Proceedings of the IXth International Symposium on Cerebral Blood Flow and Metabolism, Tokyo, Japan, May 28-June 1, 1979 (printed in Japan, Hidejima Seihanjo), Copenhagen, Munksgaard, 1979, pp. 14-15.

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23. Sokoloff, L.: Mapping of local cerebral functional activity by measurement of local cerebral glucose utilization with [^{14}C]deoxyglucose. Brain. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00886-02 LCM

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Circadian Rhythmicity of Glucose Utilization in the Rat Suprachiasmatic Nucleus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. B. Smith	Staff Fellow	LCM NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Cerebral Metabolism, NIMH

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study the autoradiographic 2-deoxy-D[^{14}C]glucose method of determining glucose utilization was applied in an attempt to demonstrate a circadian rhythm of an intrinsic property of the rat suprachiasmatic nucleus (SCN). It was found that (1) SCN glucose utilization could be entrained to a 24 hr environmental light-dark cycle and phase shifted 180° by reversing the external entraining cycle. (2) This metabolic rhythm appeared to persist in the absence of any external light-dark cues but it was arrhythmic after 10 days of constant light. (3) The effect of ambient darkness during the light phase of the entraining cycle appeared to "unmask" an underlying endogenous metabolic activity of the nucleus. (4) When animals maintained in constant darkness or constant light were exposed to either ambient light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggested that the sensitivity of the nucleus to ambient light during constant conditions may have been a function of the phase of an endogenous SCN oscillation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives. The suprachiasmatic nucleus (SCN) in the anterior hypothalamus has been proposed as the endogenous circadian oscillator in mammals. The evidence is based on the fact that a retino-hypothalamic pathway terminating in the SCN has been identified and appears to be necessary for the entrainment of circadian rhythms to the environmental light-dark cycle and that lesions of the SCN abolish a wide spectrum of overt circadian rhythms. In order to demonstrate that a directly measurable property of the SCN itself in intact (unlesioned) animals is affected by environmental light and exhibits circadian rhythmicity, the autoradiographic 2-deoxy-D-[^{14}C]glucose method was used to determine glucose utilization of rat SCN under various lighting conditions.

Methods. A total of 88 adult, male Sprague-Dawley albino rats, weighing between 300 and 400 grams each, were used in experiments conducted from October, 1977, to April, 1978. Rats were housed in individual activity-wheel cages in a room with controlled artificial lighting. During "light" phases, the cages were bathed in approximately 30 ft-c illumination from overhead fluorescent bulbs; during "dark" phases, less than 0.01 ft-c illumination was provided by two Kodak 15 watt safelights with red (1A) filters. Individual running-wheel activities were recorded.

Groups of rats were subjected to various lighting regimens before the determination of SCN glucose utilization. At least two hours after catheterization of the femoral vein and artery, animals were injected intravenously with 2-deoxy-D-[^{14}C]glucose while they were alert and restrained. After 45 min the animals were killed by an intravenous injection of sodium pentobarbital; and their brains were removed, frozen in 2-methylbutane (-40°C), and serial coronal sections 20 μm thick were cut through the region of the SCN. Sections were dried and autoradiographs prepared.

After autoradiography, the sections were stained with cresyl violet to determine the location and extent of the SCN. Optical densities of the SCN and other selected brain regions were read on a Photoscan System P-1000 densitometer (Optronics International, Inc.). Optical densities of the left and right SCN were recorded for each section along the 600 μm length of the nucleus, and mean glucose utilization of the entire nucleus was calculated.

Major Findings. The results of these experiments indicate that the rat SCN has properties consistent with a role as an endogenous circadian oscillator. The glucose utilization of the SCN can be entrained to a 24-hr environmental light-dark cycle (LD) and phase-shifted 180° by reversing the external entraining cycle (DL). Glucose utilization is high during the light phase and low during the dark phase. This metabolic rhythm appears to persist in a somewhat modified form in the absence of any external light-dark cues, i.e., during constant darkness or after bilateral enucleation. After 10 days in these constant conditions, the nucleus is metabolically active for a shorter time and at a lower level of glucose utilization during the subjective day than it had been during the light phase of either an LD or DL cycle. However, after 10 days of constant light, SCN glucose utilization appears arrhythmic and at an intermediate level. When animals in the "D" phase of an LD cycle are exposed to light, SCN glucose utilization increases from low levels to high values comparable to those observed during the "L" phase. On the other hand, dark exposure during the "L" phase results in a pattern of altered SCN glucose utilization similar to that observed during the subjective day in constant darkness or after bilateral enucleation. Dark exposure thus appears to "unmask" the endogenous activity of the nucleus. When animals in constant darkness or constant light are exposed to either light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggests that the sensitivity of the nucleus to ambient light during constant conditions may be a function of the phase of an endogenous SCN oscillation.

Proposed Course. The experimental work on this project has been terminated. Preliminary results were reported at the Ninth Annual Meeting of the American Society for Neurochemistry [Trans. Am. Soc. Neurochem. 9, 96 (1978)]. In addition, the work was presented at the Naito International Symposium on "Biorhythm and Its Central Mechanism" in August, 1978. The paper presented, *In vivo* Glucose Utilization of the Suprachiasmatic Nucleus, by W. J. Schwartz, C. B. Smith, and L. C. Davidsen, will be published as a chapter in a book of the proceedings. A manuscript of this work has been accepted for publication by the Journal of Comparative Neurology.

Publications:

1. Schwartz, W. J., Smith, C. B., and Davidsen, L. C., *In vivo* glucose utilization of the suprachiasmatic nucleus. In: Proceedings of Naito International Symposium on Biorhythm and Its Central Mechanism. (In press)

2. Schwartz, W. J., Davidsen, L. C., and Smith, C. B., *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *J. Comp. Neurol*. (In press)

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PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Extended Visual System of the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM NIMH
	M. Mishkin	Research Physiologist	LN NIMH
OTHER:	C. D. Jarvis	Research Psychologist	LN NIMH
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	K. Macko	Guest Worker	LN NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cerebral Metabolism, NIMH

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	0.75

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The deoxyglucose method is being applied to the monkey to advance knowledge regarding the parts of the brain which are involved in the processing of visual information. The involvement of clearly defined limits of cortex (largely the inferior portion of the temporal lobe) and limbic system have already been demonstrated. In future experiments it is hoped to differentiate regions involving the mental processes, memory and discrimination.

PHS-6040
(Rev. 10-76)

Project Description:

The present collaboration began last year when the deoxyglucose method was employed to map the visually responsive regions of brain outside of the well-studied geniculostriate system. Lesion effects and single cell recordings had provided evidence that an extensive region of cortex, particularly the inferior portion of the temporal lobe, is involved in processing visual stimuli. The initial experiments were carried out by preparing animals so that one hemisphere is deprived of a visual input. This was accomplished by sectioning of one optic tract and also sectioning of the corpus callosum and anterior commissures. Thus it is possible to compare the findings in the hemisphere with intact visual input with those in the hemisphere which was visually deprived. The stimulation was in the form of an illuminated, moving, geometric design. The results indicate that there is an extensive region of cortex with a lower rate of glucose utilization in the "blinded" hemisphere. This region involves the inferior temporal lobe, as was predicted, but the exact limits are delineated in a manner not heretofore possible. Also depressed in the rate of metabolism on the "blinded" side are regions within the limbic system, the limits of which are also well-defined. Similar studies carried out in the neonatal period indicate that this extra-striate, visually responsive, cortical region is functional even in early life. Its exact location, however, appears to be different. Much of the insular cortex, which is unresponsive to visual stimuli in mature animals, appears to be responsive in the neonate. These studies are being extended.

To determine what effect the nature of the visual stimulus may have on the results in the mature animal, additional experiments have been carried out. In these, changing stationary illuminated symbols are substituted for the moving geometric design. The animal, previously prepared as described above, is trained to recognize a certain symbol as a cue for a water reward. The deoxyglucose method is then carried out while the animal is performing a task in response to the visual stimulation. By comparing the results of these studies with those carried out under conditions of passive stimulation (the moving geometric design), it is hoped to determine what regions of brain may be involved in the mental process of discrimination and in the performance of motor activity involved in pressing a lever to gain the reward.

The results of a limited number of experiments were reported at the annual meeting of the Society for Neuroscience in St. Louis in November, 1978. The series has been enlarged through the year, and much of the data is now being analyzed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00888-02 LCM

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Maturation of Cortical Function in the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM NIMH
	P. Goldman	Research Physiologist	LN NIMH

COOPERATING UNITS (if any)
Laboratory of Neuropsychology, NIMH

LAB/BRANCH
Laboratory of Cerebral Metabolism, NIMH

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments have been carried out on this project by Dr. Goldman and her associates; we have served only as consultants. The project is still in a developmental state, and no definitive results have yet been obtained.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00900-23 LCM

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Myelin and Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Martenson	Research Chemist (Biochem.)	LCM	NIMH
Other:	G. E. Deibler	Chemist	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The function of myelin basic protein (BP) in vivo is unknown except for its role as a structural protein in myelin. It is susceptible to phosphorylation both in vitro and in vivo. Isolated BP contains only 2 phosphorylated residues, Thr-96 and Ser-153. Yet in vitro studies with ^{32}P-labeled ATP show that other residues, Ser-12, Thr-35, and Ser-56, are readily labeled when myelin-bound BP is phosphorylated with myelin-bound protein kinase. The high degree of specificity of these reactions suggests that a study of phosphorylation in vivo might throw some light on the function of BP.

PHS-6040
(Rev. 10-76)

Project Description:

In order to learn more about the function of the myelin basic protein (BP), initial steps have been taken to study the protein's in vivo phosphorylation. There are several interesting aspects of BP phosphorylation that should be noted: 1) In the isolated protein about 20% of the molecules are phosphorylated. The overwhelming majority of these contain a single phosphate per molecule, either as phosphothreonine (residue 96) or phosphoserine (residue 163); the remainder contains phosphate at both sites. 2) When myelin-bound basic protein is phosphorylated in vitro with [γ-^{32}P]ATP and myelin-bound protein kinase, acid stable ^{32}P is found principally on Ser-12, Thr-35, and Ser-56; no ^{32}P is found on Thr-96 or Ser-163. 3) When basic protein is not exposed to acid conditions during isolation, it has been reported that phosphorylation of arginyl and histidyl residues also occurs. Phosphoarginine and phosphohistidine are extremely labile at acid pH, conditions nearly always used in the isolation of the basic protein. 4) Phosphorylation of soluble (isolated) BP with soluble protein kinase results in the phosphorylation of many more serine and threonine residues than detected in 1) or 2) above. 5) At least some of the acid-stable basic protein phosphate groups turn over fairly rapidly in vivo.

The first step in this study was to devise a procedure for the fragmentation of the basic protein into peptides that could be readily separated and identified so that incorporated ^{32}P could be localized to definite regions of the polypeptide chain. Rabbit basic protein was digested with pepsin at pH 6.0 under controlled conditions so that either smaller or larger peptides could be produced in high yield. The following small peptides have been isolated in pure form, and their relative mobilities on acrylamide gels have been determined. They were identified unequivocally by amino acid analysis, C-terminal analysis, and tryptic peptide mapping: peptides (88-151), (88-109), (110-151), (152-168), (1-14), (15-44), (1-44), and (45-87).

The results of these analyses are consistent with the sequence of the rabbit protein previously proposed by other investigators, with one exception. This is the sequence of residues 107-110, which we have established to be Thr-Val-Leu-Ser, a sequence unlike that found in other myelin basic proteins.

Of particular importance is the fact that peptide (152-168) is generated very rapidly upon peptic hydrolysis and is well separated from all other peptides during gel electrophoresis. This peptide, which contains Ser-163, also contains a methionyl residue, which can be labeled in vivo with L-[methyl-^{3}H]-methionine to establish coincidence of the ^{32}P- and ^{3}H-containing peptides. Peptide (88-109) is also produced in high yield and is well separated from the other peptides. This peptide, which contains Thr-96, also contains methylated arginine, which could likewise be specifically labeled in vivo with L-[methyl-^{3}H]-methionine in a double labeling experiment.

The larger fragments include peptides (1-151), (45-168), (45-151), (1-87), and (88-168). These are currently being purified and identified.

Peptide (45-151) is of particular interest, since it is nearly identical with the fragment of the bovine basic protein produced by in situ proteolysis that has been claimed by others to have fibroblast growth factor activity.

Both large and small peptides are valuable for the delineation of sequences that induce allergic encephalomyelitis as well as those which induce antibody production. In addition, since the basic protein has very little tertiary structure, the wide variety of peptides of different regions and of different lengths should permit the identification by circular dichroism of those regions which assume a helical secondary structure upon interaction with acidic lipids or anionic detergents.

Basic protein can be extracted directly from isolated, lyophilized myelin with 0.5 M NH_4HCO_3 at pH 8.4 without exposure to acid. This will permit analyses for phosphohistidine and phosphoarginine as well as provide "undenatured" material for physicochemical studies and a source for generation of possible acid-denaturable growth factors. The presence of fibroblast growth factor activity of various BP preparations and fragments (highly purified as well as partially purified) is currently under investigation.

Publications:

Martenson, R. E.: The use of gel filtration to follow conformational changes in proteins. Conformational flexibility of bovine myelin basic protein. J. Biol. Chem. 253: 8887-8893, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00901-24 LCM

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immunologic Reactivity of Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
Other:	J. R. Richert	Guest Worker	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. C. Alvord, Jr.,University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transfer of EAE with BP-sensitized Lewis rat spleen cells has been used to study immune mechanisms in the induction and suppression of EAE. Specifically, we have attempted to define the conditions under which culture with specific antigen activates BP-sensitized spleen cells as well as those conditions which might generate antigen-specific suppressor cells in culture.

PHS-6040
(Rev. 10-76)

Project Description:

Lewis rats are sensitized with basic protein (BP) in complete Freund's adjuvant (CFA) and their spleens removed aseptically on day 11 or 12 for preparation of short-term cell cultures. We previously reported that cells cultured for 72 hours in the presence of BP 10 μg/ml were capable of transferring experimental allergic encephalomyelitis (EAE) (10^7 cells/recipient). A more detailed examination of culture conditions required for successful transfer has shown that the minimum time in culture is between 4 and 12 hours and the minimum concentration of BP required is 10 ng/ml. Optimal results are obtained with 1 μg BP/ml.

The effect of culture conditions on cell proliferation indicates a peak response with 1 μg BP/ml after 96 hours culture. The marked difference in time requirements suggests that proliferation in vitro is not a prerequisite for successful transfer.

The use of NH_4Cl to lyse red blood cells prior to culture abolished the proliferative response (measured by increased $[^3H]$-TdR uptake) but did not affect transfer adversely. The same results were obtained after lysis with distilled water. These experiments also suggested that proliferating cells were not responsible for transfer. Results of other studies (with BUdR and light, mitomycin C, and fractionation on BSA gradients) were not consistent with this interpretation. Our present conclusion is that cellular proliferation in the recipient animals is essential for successful transfer.

Our attempts to generate antigen-specific suppressor T-cells in vitro with rat spleen cells have so far been unsuccessful.

The spleen cell transfer system appears to be unique to the rat (we were unable to transfer EAE with sensitized guinea pig spleen cells under any circumstances). Conconavalin A activation (reported by others) also appears to be a unique property of rat spleen cells. In both species BP-sensitized lymph node cells (LNC) are capable of transferring EAE and the LNC of both species can be "activated" (transfer enhanced) by culture with specific antigen. Thus, the system offers a unique opportunity to study species differences which are important in the induction and suppression of EAE.

Publications:

Richert, J. R., Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Adoptive transfer of experimental allergic encephalomyelitis: incubation of rat spleen cells with specific antigen. J. Immunol. 122: 494-496, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00902-14 LCM

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. F. Driscoll	Guest Worker	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

National Multiple Sclerosis Society
E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Knowledge of pathogenetic mechanisms involved in the induction of EAE has been gained indirectly from studies in the whole animal. In vitro cell studies (reported last year) indicated that proliferation in culture of BP-sensitized cells in the presence of antigen correlates well with the disease state but this proliferation is not induced exclusively by the region of the molecule responsible for disease induction. These studies led to an important observation that culture with antigen in vitro markedly enhanced (~100-fold) the ability of BP-sensitized cells to transfer EAE. With the small number of cultured cells required for transfer (0.5-1 x 10^7) we have been able to fractionate the BP-cultured cells into an active and an inactive population.

PHS-6040
(Rev. 10-76)

Project Description:

The induction of experimental allergic encephalomyelitis (EAE) with myelin basic protein (BP) in complete Freund's adjuvant (CFA) is mediated by thymus-dependent lymphocytes (T-cells). Direct demonstration of antigen-specific T cells is impossible because of their failure to bind detectable amounts of antigen (in contrast to specific B cells). Indirect tests for the presence of antigen specific T-cells are based on antigen mediated proliferation or production of lymphokines (in vitro) or delayed hypersensitivity reactions, e.g. skin test (detected in vivo). A more direct test is the killing of target cells by cytotoxic T cells. None of these reactions with the possible exception of cytotoxicity has yielded useful information for analyzing the immune response. In the case of EAE our previous experience with in vitro proliferative response convinced us this was not a dependable assay for EAE effector cells. (Proliferation induced with fragments of the BP molecule did not correlate with the ability of the fragments to induce disease.)

The only aspect of cell mediated immunity to BP which is critical to the study of autoimmune mechanisms is the induction of disease. The detection of cells specifically programmed for induction of EAE is easy in principle--donor animals are sensitized with BP in CFA and their lymph node cells transferred to normal recipients. But such a large number of cells, 10^9/recipient, is required that the cells cannot be fractionated in vitro.

As an extension of our study of the proliferative response of peritoneal exudate cells (PEC) to specific antigen (reported last year), we transferred some of these cells to test their activity in recipients. (We had already observed that 5×10^8 PEC unincubated cells induced only mild disease.) In contrast to those results, we discovered that as few as 10^7 incubated cells induced acute disease with accelerated onset.

Using this technique, we have followed the course of disease development in the animal and attempted to elucidate the role of various cell types in disease induction. Furthermore, since it is unknown why cell mediated immunity against BP leads to disease, an understanding of how culture with antigen leads to enhanced disease transfer might clarify the mechanism(s) by which cells interact with the target organ.

An analysis of the parameters of enhanced transfer has shown that 1) such cells appear in the PEC as early as 7 days after sensitization. 2) BP activation requires a finite time in culture--maximal reactivity is obtained after 18 hours in culture with antigen. This is too short a time for proliferation in culture but the fact that mitomycin C treatment destroys the ability of the cultured cells to transfer disease suggests that proliferation in the recipient is essential to enhanced transfer. 3) Cells responsible for transfer are found in the low density "blast cell" population when the total cell population is separated on density gradients.

Since the cells can be separated into fractions positive and negative for EAE transfer, fractions negative for transfer are being tested for the presence of suppressor cells. While the data are preliminary, it appears that recipients of these cells display altered disease induction when sensitized with BP/CFA.

Publications:

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Transfer of experimental allergic encephalomyelitis with guinea pig peritoneal exudate cells. Science 203: 547-548, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00903-02 LCM

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Preparation and Identification of Peptides Derived From Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. Deibler	Chemist	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Myelin Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myelin basic protein (BP) presents a unique opportunity for a detailed study of the relationship between structure and biological activity--it has been completely sequenced and there is an impressive backlog of information on its encephalitogenic activity in several species; its ability to induce and bind antibody and its ability to bind other biochemical compounds or structures, specifically membrane constituents.

In order to study the relationship between structure and activity, we have prepared several well-characterized fragments of the protein by controlled proteolytic digestion and have purified several synthetic peptides prepared for us by Dr. Janis Young, Space Sciences Laboratory, University of California, Berkeley. Our purpose is to test the ability of these peptides to replicate the biological activity of the protein. By using fragments from different regions of the protein one can "map" active sites and define the minimal structural requirements for activity.

PHS-6040
(Rev. 10-76)

Project Description:

Last year we reported preliminary bioassay data on the activity of synthetic peptides related to the guinea pig encephalitogenic site. The peptides were partially purified by cycling through Sephadex G-25 and characterized by amino acid analyses and high voltage electrophoresis (HVE). Although the amino acid analyses agreed with the theoretical compositions within the 10% error of this procedure, all peptides exhibited more than one spot on HVE. Two of the peptides were further purified on high pressure liquid chromatography: 1) the shortest peptide which comprised the active site (nonapeptide, 113-121 and 2) the longest peptide of the group which included the nonapeptide sequence plus two additional N-terminal residues and 7 C-terminal ones (111-128).

From the bioassay data we can conclude: 1) The BP-nonapeptide has definite encephalitogenic activity but the disease induced is milder, even with a 50-fold molar excess, than that induced by whole BP. 2) Extending the nonapeptide either by 2 residues on the N-terminal end or by 7 residues on the C-terminal end does not enhance its activity. 3) If the additional residues in 2) are added simultaneously, i.e., at both ends of the nonapeptide chain, the specific activity is markedly enhanced--approaching that of BP.

Diagrammatically this can be expressed as follows:

Nonapeptide*
NH_2/- - - - - - - - -/COOH

Equivalent (mild) activity NH_2- -/- - - - - - - - -/COOH

NH_2/- - - - - - - - -/- - - - - - - COOH

Enhanced activity NH_2- -/- - - - - - - - -/- - - - - - - COOH

* Each hyphen represents an amino acid residue.

One explanation of the enhanced activity obtained with the longest peptide is that the additional residues enabled the nonapeptide to assume its "native" conformation more readily--i.e., the conformation of the nonapeptide in the whole BP molecule and that this conformation was optimum for encephalitogenic activity. We hope to be able to correlate the *in vivo* (encephalitogenic) activity of these peptides with their ability to replace BP in its reaction with specifically sensitized cells *in vitro*.

In a related study of guinea pig BP-derived peptide fragments isolated by Staphylococcus aureus V8 protease digestion, peptides corresponding to residues 89-127, 89-119, 120-169, and 128-169 were purified and characterized. Peptide 89-127 was fully active at 1 nmole in guinea pigs (equivalent to 1 nmole whole BP); the other fragments were inactive. Note that the active synthetic fragment 111-128 duplicates the last 17 residues of 89-127.

No signs of EAE were seen in Lewis rats injected with either 1 or 10 nanomoles of each of these "V-8" peptides. These results failed to confirm earlier results obtained with bovine BP-derived peptides (pepsin digests) which suggested a weak encephalitogenic site between residues 89 and 115. Higher levels of these V8 protease fragments will be tested after they have been further purified.

All of these fragments will be tested eventually for 1) their ability to enhance transfer of EAE; 2) their ability to prevent or suppress disease; and 3) their ability to induce antibody or to bind antibody induced with whole BP. Thus, it should eventually be possible to map the immunologic reactivity of the entire molecule.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00931-06 LGCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on the Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH
Other:	Y. S. Im	Visiting Fellow	LGCB NIMH
	A. B. Guranowski	Visiting Associate	LGCB NIMH
	G. L. Kunz	Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

J. P. Bader, Laboratory of Tumor Virus Genetics, NCI

E. J. Leonard, Laboratory of Immunobiology, NCI

E. Schiffmann, Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-5/6	5/6	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

After screening a large number of chemicals that are either analogs of adenosine or analogs of S-adenosyl-L-homocysteine (AdoHcy) that are modified in the base, ribose, sulfur or the homocysteinyl moiety, two most promising inhibitors of AdoHcy hydrolase have been found. They are 3-deazaadenosine (deaza-Ado) and 5'-deoxy-5'-(isobutylthio)-3-deaza-adenosine (deaza-SIBA). Deaza-Ado is unique in being both a substrate and also inhibitor of AdoHcy hydrolase. Deaza-Ado and other purine analogs, such as Ara-A, are also irreversible inhibitors of AdoHcy hydrolase. Both deaza-Ado and deaza-SIBA exert a broad spectrum antiviral activity against RNA and DNA viruses, as well as being able to prevent cellular transformation induced by oncogenic viruses. Both chemicals exhibit antimalarial activity against *Plasmodium falciparum* in culture. Deaza-Ado is a potent inhibitor of chemotaxis by neutrophil leukocytes, and phagocytic function by macrophages. When administered *in vivo* to rats, deaza-Ado caused a marked inhibition in the methylation of liver lipids, with concomitant reduction in the urinary excretion of 3-methoxy-4-hydroxy-mandelic acid and creatine biosynthesis in the liver.

PHS-6040
(Rev. 10-76)

Project Description:

Adenosylhomocysteine (AdoHcy) is the product of biological methylation reactions that utilize adenosylmethionine (AdoMet) as the methyl donor. AdoHcy itself was first isolated and characterized by Cantoni and Scarano. As far as is known, the main pathway for the metabolism of AdoHcy in eukaryotes is its hydrolysis to adenosine and L-homocysteine by an enzyme, AdoHcy hydrolase, first described in rat liver by De la Haba and Cantoni. The reaction is reversible, with equilibrium far in the direction of synthesis:

$$\text{AdoHcy} \underset{H_2O}{\overset{\text{AdoHcy hydrolase}}{\rightleftharpoons}} \text{Adenosine + L-Homocysteine}$$

Many biological substances are methylated by AdoMet, such as biologenic amines, proteins, amino acids, lipids, carbohydrates, histones, myosine, DNA, and RNA. Interestingly as a rule, almost all of the enzyme methylases involved are sensitive to product inhibition by AdoHcy.

Much attention has been focused on AdoHcy per se as a biological regulator of methylation reactions, and many analogs of AdoHcy have been synthesized, and their efficacies as inhibitors of biological methylations studied. We are combining a study of the molecular properties of AdoHcy hydrolase and its regulation by effectors, with a detailed pharmacological exploration of its perturbation *in vivo* and *in vitro* by potent inhibitors, e.g. 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine. The biological consequences of such a perturbation may be of paramount medical importance as well as yielding knowledge on the biochemical interplay between AdoHcy and AdoMet.

AdoHcy hydrolase was prepared from beef liver by crystallization. It was found that AdoHcy hydrolase can be irreversibly inactivated by a large number of purine analogs. The irreversible inhibition of AdoHcy hydrolase by some purines could be due to a suicidal mechanism. Among the more potent irreversible inhibitors are 2-chloroadenosine, Ara-A, and aristeromycin, in that order. After incubation with [^{14}C]adenosine and [^{3}H]Ara-A, tight binding of these ligands onto the enzyme can be demonstrated by gel electrophoresis. The enzyme activity lost after incubation with these purine analogs cannot be restored even after electrophoresis. It was found that AdoHcy hydrolase exhibits phenomenon of half-site reactivity with regard to ligand binding with adenosine, i.e. two moles of adenosine bound per mole of enzyme, or half a mole of adenosine bound for subunit of AdoHcy hydrolase. The K_D (dissociation constant) is about 1.4×10^{-9} M. Irreversible inactivation of AdoHcy by some purine analogs is probably a result of a suicidal mechanism, as suggested by our observations that adenine is cleaved from adenosine and Ara-A and 3-deazaadenine from 3-deazaadenosine by AdoHcy hydrolase. However, this suicidal inactivation of AdoHcy hydrolase by Ara-A can be completely prevented by the substrate, AdoHcy itself.

An exciting aspect of the utility of AdoHcy analogs begins to develop in the field of neuroscience. In collaboration with Dr. Marshall Nirenberg and his co-workers, it was found that both 3-deazaadenosine (DZA) and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine (deaza-SIBA) exert profound inhibition of synaptic muscle responses in tissue culture. The tissue culture system consists of neurons from 8-day chick embryo retina grown in culture with rat striated muscle cells for 24 hours to form synapses. After treating the cultures for 2 hours with either analog, the number of muscle responses/min in these synapses was inhibited up to 95% by deaza-SIBA ($I_{50} = 3 \times 10^{15}$ M), and DZA ($I_{50} = 1.5 \times 10^{-5}$ M). Homocysteine thiolactone, 5'-deoxyadenosine and tubercidin, which do not increase levels of AdoHcy or inhibit methyltransferases, had no effect. Deaza-SIBA inhibited the muscle responses with a half-time of 3.5 min. No inhibition of the number of synapses formed was seen. Inhibition of the number of muscle responses/min by DZA was reversed within 2 hours after removal of the compound. Furthermore, homocysteine thiolactone potentiated the inhibition of DZA by 6-fold when the two compounds were added together. These results strongly suggest that a methyltransferase-mediated reaction occurs during neurotransmission, and also point to research possibilities in neuroscience quite exciting to come.

We continue to study the effects of DZA, and deaza-SIBA as antiviral agents in tissue culture systems. Both DZA, and deaza-SIBA exert a broad spectrum antiviral activity against RNA and DNA viruses: Rous sarcoma virus, Gross murine leukemia virus, Sindbis, Newcastle disease virus, vesicular stomatitis, influenza virus, Simian virus 40, herpes simplex, and HL-23 virus. Moreover, both DZA and deaza-SIBA can prevent cellular transformation induced by oncogenic viruses and DZA itself is able to reverse the malignant transformation induced by Rous sarcoma virus and to restore normal growth patterns.

An interesting facet of the usefulness of DZA and deaza-SIBA is their ability to elucidate immunological responses by leukocytes. It is postulated that cell (chemotaxis) migration toward a gradient responses involves methylations of the cell membranes. DZA is the most potent purine analog that can inhibit chemotaxis by neutrophil leukocytes, with an I_{50} of 7×10^{-6} M. Other analogs, such as ^{6}N-methyladenosine, purine riboside, and deaza-SIBA can also inhibit chemotaxis. Another aspect of cellular motility is phagocytosis by macrophages. Again, DZA can inhibit phagocytosis as low as 1 μM. These data taken together, suggest that DZA can perturb methylation reactions of cell membranes, by altering the AdoMet/AdoHcy ratio. This hypothesis is borne out by the findings that AdoHcy is drastically increased in these cells along with the appearance of 3-deaza-AdoHcy after incubation with DZA.

The potential use of DZA and deaza-SIBA as biochemical as well as pharmacological probes of cellular functions is unlimited and will remain quite illuminating yet.

Publications:

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Bader, J.P., Brown, N.R., Chiang, P.K., and Cantoni, G.L.: 3-Deazaadenosine an inhibitor of adenosylhomocysteine hydrolase inhibits reproduction of Rous sarcoma virus and transformation of chick embryo cells. Virology 89: 494-505, 1978.

Leonard, E.J., Skeel, A., Chiang, P.K., and Cantoni, G.L.: The action of the adenosylhomocysteine hydrolase inhibitor, 3-deazaadenosine, on phagocytic function of mouse macrophages and human monocytes. Biochem. Biophys. Res. Commun. 84: 102-109, 1978.

Cantoni, G.L., Richards, H.H., and Chiang, P.K.: Inhibitors of S-adenosylhomocysteine hydrolase and their role in the regulation of biological methylation. In Usdin, E., Borchardt, R.T., and Creveling, C.R. (Eds.): Transmethylation. New York, Elsevier, 1979, pp. 155-164.

Chiang, P.K., Venkatasubramanian, K., Richards, H.H., Cantoni, G.L., and Schiffmann, E.: Adenosylhomocysteine hydrolase and chemotaxis. In Usdin, E., Borchardt, R.T., and Creveling, C.R. (Eds.): Transmethylation. New York, Elsevier, 1979, pp. 165-172.

Chiang, P.K., and Cantoni, G.L.: Perturbation of biochemical transmethylations by 3-deazaadenosine *in vivo*. Biochem. Pharmac., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00932-07 LGCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Muscle Differentiation in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
Other:	B. W. Uhlendorf	Res. Microbiologist	LGCB NIMH
	J. K. Chen	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

R. S. Adelstein, Chief, Sect. Molecular Cardiology, NHLI

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-1/3	2-1/3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myogenesis *in vitro* has been much studied as a model of cellular differentiation. The formation of multinucleated non-proliferating electrically active and contractile myotubes by fusion of undifferentiated, proliferating myoblasts is accompanied by the appearance of muscle specific proteins, such as creatine kinase and myosin, and by the arrest of DNA synthesis. By manipulating the environmental conditions of a cloned line of myoblasts we are able to study these morphological and biochemical events. In addition, we hope to ellucidate the nature of the unknown cue(s) that signal the cell to proceed along the path of differentiation rather than the path of continued proliferation.

PHS-6040
(Rev. 10-76)

Project Description:

We are studying the basic biological process of cellular differentiation. Our experimental object has been L5/A10, a line of cells cloned in this laboratory from the L5 line of rat myoblasts. More recently, we have compared the growth and differentiation of this line with that of the much used L8 line of myoblasts, also of rat origin. An important and useful characteristic of the L5/A10 cells is that they can be programmed by manipulation of the composition of the culture medium to either proliferate to very high densities of undifferentiated mononuclear cells or to differentiate into multinucleated myotubes. We have found that in medium containing 1% serum both L5/A10 and L8 cells stop growing after four days and then over the next three to four days fuse and produce comparably high levels of creatine kinase (CPK) and myokinase (MK). In medium containing 10% serum, whereas the L8 cells fuse and produce CPK (but not increased levels of MK), the L5/A10 cells neither fuse nor produce increased levels of either enzyme. A consequence of the difference in behavior of the two cell lines in 10% serum is that the L5/A10 cells can be serially subcultured many times without loosing their ability to differentiate, while the L8 cells loose their ability quickly and must be recloned periodically.

In order to further elucidate the nature of the cue(s) to differentiation, we also studied the effects of several serum fractions, fibronectin, delipidated serum, various endoglycosidases, bromouracildeoxyribose, high calcium levels, Con A, insulin, 3-deazaadenosine, dimethylsulfoxide and dimethylformamide on one or more of the following differentiating systems: myogenic, chondrogenic, erythropoietic, melanocyte differentiation and adipocyte conversion.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00933-05 LGCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Study of Adenosylmethionine Synthetase and Enzymes of Transmethylations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH
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COOPERATING UNITS (if any)

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LAB/BRANCH
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular properties of AdoMet synthetase and enzymes of transmethylation are being characterized. Guanidoacetate methyltransferase has been purified. Guanidoacetate methyltransferase has been purified about 140-fold from pig liver. Polyacrylamide gel electrophoresis of the purified enzyme showed four protein bands, each of which is associated with guanidoacetate methyltransferase activity. During gel electrophoresis at pH 3 in 8 M urea, guanidoacetate methyltransferase migrated as a single component. The molecular weight of the purified guanidoacetate methyltransferase was estimated to be 31,000 by sodium dodecyl sulfate-gel electrophoresis, which also showed only one protein component with guanidoacetate methyltransferase activity. Guanidoacetate methyltransferase is inhibited by adenosylhomocysteine, 3-deazaadenosylhomocysteine, and sinefungin with K_i values of 16 μM, 39 μM, and 18 μM, respectively.

PHS-6040
(Rev. 10-76)

Project Description:

Adenosylmethionine (AdoMet) a product of ATP plus methionine formed by the enzyme AdoMet synthetase, is one of the most versatile compounds in biology. It can function as a methyl donor in a very large number of transmethylation reactions, such as the methylation of biogenic amines, lipids, DNA, RNA, peptides myosin, amino acids, membranes, and myelin. Depending on the particular reaction or product involved, a biological reaction can either be initiated, activated or terminated after the transmethylation reaction. It can also function as a donor of a 3-amino-3-carboxypropyl group, of the amino group of homocysteinyl moiety, and after decarboxylation, of a propylamine side chain. In addition, it is cleaved enzymatically to generate thiomethyladenosine and homoserine lactone. Moreover, it can function as an allosteric effector in a variety of reactions.

For many years, there has been considerable interest in the biochemistry and pharmacology of biological transmethylation in the field of neuropsychiatry, which has been largely due to the fact that many natural or synthetic substances produce a variety of psychotropic effect, including hallucinations or other reactions that also occur in psychotic illness, are methylated amines. It has been suggested in the early fifties that abnormal transmethylation of an endogenous amine, possibly dopamine, might produce a psychomimetic compound like mescaline. Circumstantial evidence consistent with this hypothesis was the observation that methionine, when combined with an inhibitor of monoamine oxidase, could lead to striking and reversible exacerbations of the psychotic symptoms of chronic schizophrenia patients without inducing signs of delirium. There have been reports that AdoMet levels in the blood of acutely psychotic, but not chronically psychotic, schizophrenic subjects are low, indicating possibly either an excessive utilization or deficient production of AdoMet. An interesting study recently reported that intravenously injected AdoMet could exert clinically useful psychotic effects in depression. Moreover, initial findings of altered methionine-methyl metabolism in the leukocytes of schizophrenic patients have been reported, suggesting an abnormality in transmethylation process and/or an alteration in the metabolic pathway for the oxidation of this methyl group to CO_2. The fact that this abnormality was demonstrated in leukocytes suggests that the metabolic disorder associated with schizophrenia may be manifested in the general metabolism of the body. If these preliminary findings are confirmed by further studies, they not only will support the hypothesis of a transmethylation defect as a biochemical basis for schizophrenia, but also suggest a new approach to the study of this disease.

In view of the undisputed importance of AdoMet in transmethylation, Dr. Chiang continues to study the enzyme AdoMet synthetase, which produces AdoMet. More potent inhibitory analogs of methionine are still explored. The important discovery by Dr. Chiang of the two forms of AdoMet synthetase has now been confirmed by a research team in France, and by others for the enzyme from liver.

Since the demand for the labile methyl groups from the methionine source is determined largely by the requirement for creatine synthesis and the enzyme responsible for the synthesis of creatine, guanidoacetate methyltransferase (GAA-MT), has received little attention in spite of its importance in methyl balance, we carried out the purification and detailed study of GAA-MT.

GAA-MT has been purified 140-fold from pig liver. Polyacrylamide gel electrophoresis of the purified enzyme showed from protein bands, each of which is associated with GAA-MT activity. When subjected to gel electrophoresis at pH 3 in 8 M urea, GAA-MT migrated as a single component. The molecular weight of the purified GAA-MT was estimated to be 31,000 by sodium dodecyl sulfate gel-electrophoresis, which also showed only one protein component with GAA-MT activity. This molecular weight is in agreement with that estimated by Sephadex G-75 chromatography. GAA-MT is inhibited by adenosylhomocysteine, 3-deazaadenosylhomocysteine, and sinefungin with K_i values of 16 μM, 39 μM and 18 μM, respectively, whereas the K_m for AdoMet was 49 μM.

The strong inhibitory potency of adenosylhomocysteine and 3-deazaadenosylhomocysteine on the enzyme GAA-MT *in vitro* is thus constent with the observation that after the administration of 3-deazaadenosine in rats resulted in about 50% reduction in the level of creatine in rat livers. The reduction in the creatine level was assumed to be due to inhibition of creatine biosynthesis in the liver as a result of the increased intracellular concentration of AdoHcy, the formation of a large amount of 3-deaza-AdoHcy, and the accompanying changes in the ratio of AdoMet/AdoHcy or more specifically, AdoMet/(AdoHcy + 3-deaza-AdoHcy).

Chemotaxis by neutrophils is a tactile movement of a population of leukocytes towards a target, and normally the target is an invading microorganism to the host. The neutrophils respond to a chemical stimulus gradient towards the target. During the sensory transduction process, the membranes of the neutrophils undergo busy and intense methylation and demethylation processes. The methylation and demethylation processes can either act as signals or as results of cell motility. By the use of 3-deazaadenosine, which inhibits the adenosylhomocysteine hydrolase to produce an accumulation of adenosylhomocysteine (a methylation inhibitor), neutrophil chemotaxis could be blocked. The inhibition in chemotaxis can be correlated with blocks in transmethylation processes. The physiological roles played by transmethylation in cell motility will be quite exciting to come.

Publications:

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Schiffmann, E., O'Dea, R.F., Chiang, P.K., Venikatasubramanian, K., Corcoran, B., Hirata, F., and Axelrod, J.: Role for methylation in leukocyte chemotaxis. In Atkinsia, D. (Ed.): ICU-UCLA Symposium on Covalent and Non-Covalent Modulation of Protein Function. New York, Academic Press, in press, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00934-07 LGCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on the Biochemical Basis of Narcotic Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Knight	Chemist	LGCB	NIMH
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	K. C. Rice	Research Chemist	LC	NIAMDD
	S. Rapoport	Chief, Lab. of Neurosciences	LN	NIA

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Laboratory of Neurosciences, NIA

LAB/BRANCH

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SECTION

Section on Proteins

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A cell line of neural origin (NG108-15) which carries many opiate receptors is used to study the mechanism of action of morphine and morphine-like peptides (endorphins). These materials exert a receptor mediated dual regulation of adenylate cyclase; a rapid inhibition of activity upon which is superimposed a subsequent increase in the amount of total expressible enzyme activity. This, homeostatic, mechanism may account for tolerance to and dependence upon the opiates. Incubation of NG108-15 cell membranes with opioids for 60 minutes leads to a compensatory increase in adenylate cyclase activity. The membrane system reproduces the essential features of opioid actions in the living cell, and provides a simple, rapid and quantitative measure of physical dependence liability of opioids, as well as opportunities to study the mechanism of dependence in molecular detail. In related studies: rat brain peptidases have been characterized, one of which may play a role in the biosynthesis of the opioid peptide methionine enkephalin; opioid peptides derived from food proteins, the exorphins, have been further characterized; and it has been found that opioid peptides will penetrate the blood-brain barrier at easily measurable rates.

PHS-6040
(Rev. 10-76)

Projection Description:

In our studies of the mechanism of morphine action, we have continued to exploit the properties of the neuroblastoma x glioma cell line NG108-15. This hybrid cell has a large number of opiate receptors which are functionally coupled to the enzyme, adenylate cyclase, as inhibitors. These cells become tolerant to and dependent upon morphine and other opioids as a result of being cultured in their presence for 12 hours or longer. This addictive process is a homeostatic mechanism which serves to maintain cellular cyclic AMP levels near their normal values. The acute effects of opiates are to inhibit adenylate cyclase and thereby rapidly lower cAMP levels; the chronic adaptation is due to a gradual expansion in the amount of enzyme activity which serves to maintain normal cAMP levels even in the continued presence of morphine. The increased enzyme activity is due, at least in part, to changes in the regulatory properties of the pre-existing enzyme rather than to an increase in the number of enzyme molecules in the cell. We have now developed a cell free preparation which acquires the biochemical attributes of opioid tolerance and dependence. Membranes, prepared from neuroblastoma x glioma hybrid NG108-15 cells, contain functional opioid receptors which remain coupled, as inhibitors, to adenylate cyclase. Incubation of these membranes with opioids for 30-60 minutes in the presence of Mg·ATP and a soluble protein fraction leads to the compensatory increase in adenylate cyclase activity. The membranes thus become tolerant to the inhibitory action of opioids and exhibit dependence as well since a naloxone challenge allows the expression of a supernormal amount of enzyme activity. This process appears to reproduce all of the essential features of opioid actions in the living cell. Because of the ease of manipulation of the cell free system, it becomes possible to examine features of the mechanism of acquisition of opioid tolerance and dependence in molecular detail. Induction of dependence in this cell free system requires that more than half of the opioid receptors be occupied with opioid agonists. Thus, mixed agonists such as cyclazocine, nalorphine and buprenorphine do not lead to dependence in this system, in agreement with results obtained with animals. It has been found that the degree of dependence induction in the membranes is closely correlated to the agonist intrinsic activity of a large series of opioids. Thus this system provides a simple, rapid and quantitative test for physical dependence liability of opioids. The direct quantitation of dependence liability has not heretofore been possible.

The opioid peptides behave in qualitatively identical fashion in the neuroblastoma x glioma hybrid cell and cell membrane systems as do the alkaloid opiates such as morphine. Thus, tolerance and dependence can be observed in whole cells and in cell free systems with the opioid peptides methionine enkephalin and β-endorphin. Indeed, enkephalin shares with the alkaloid, etorphine, the property of being the most efficacious inducer of opioid dependence which we have found.

Our interest in opioid peptides has led us to search for, and find, enzyme activities in brain which may play a role in the biosynthesis of enkephalin from larger precursors. We have developed an amphiphilic substrate, based upon the peptide β-lipotropin (61-69) which is tagged with ^{14}C by conversion of lysine 69 to ^{14}C-homoarginine. The radioactive substrate is quantitatively adsorbed to dextran-coated charcoal whereas the radioactive tetrapeptide product of enkepahlin production remains in solution and can easily be measured. With this assay, two enzyme activities, one soluble and the other particulate, have been found in brain which produce enkephalin. The specificity of the cleavage was confirmed by chemical analyses. Based upon regional distribution and kinetic considerations, we believe that the particulate activity may well play a role in enkephalin biosynthesis whereas the soluble activity may function as a degradative enzyme. These enzymes are currently being purified to allow more detailed studies of their specificity. The assay procedure developed here should be of general applicability in the study of specific peptidases.

Studies on exorphins, opioid peptides derived from pepsin digests of food proteins, have continued. Highly purified exorphins have been prepared from pepsin digests of wheat gluten and bovine α-casein. The gluten exorphin appears to be extraordinarily potent in a number of opiate assay systems whereas the peptide isolated from casein digests is much less potent but present in much larger amounts. The physiological significance of such, food derived, opioid peptides is still an open question. Recent studies which we have carried out show, however, that opioid peptides like enkephalin and the endorphins readily pass the blood-brain barrier. Thus the exorphins, which appear to share the lipophilicity of the endogenous opioids would almost certainly be able to reach the brain from the bloodstream. This work also raises the possibility that some of the endogenous peptides found in the brain may be synthesized elsewhere.

In the course of our studies of the exorphins we found that extracts of many proteins, including wheat gluten, contain a substance which has activities opposed to those of the opiates in both the electrically stimulated mouse vas deferens and the NG108-15 adenylate cyclase assays. We have identified this substance as adenosine, an agent which has many potent pharmacological and physiological activities. Although the gastrointestinal tract is normally very rich in adenosine deaminase, an enzyme which inactivates adenosine, it is conceivable that in special circumstances dietary adenosine may play a role in pathological processes.

Publications:

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Jacquet, Y.F., Klee, W.A., and Smyth, D.G.: β-Endorphin: modulation of acute tolerance and antagonism by endogenous brain systems. Brain Res. 156: 396-401, 1978.

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Klee, W.A.: Drug actions mediated by changes in adenylate cyclase activity. In Sharp, C.W., and Abood, L.G. (Eds.): Biochemical Mechanisms of Drug Action on Neuronal Membrane Systems. New York, Alan R. Less, Inc., 1979, pp. 219-225.

Klee, W.A.: Molecular mechanisms of opioid action in neuroblastoma x glioma cells. In Beers, R.F., and Bassett, E.G. (Eds.): Mechanisms of Pain and Analgesic Compounds. New York, Raven Press, 1979, pp. 361-372.

Reden, J., Reich, M.F., Rice, K.C., Jacobson, A.E., Brossi, A., Streaty, R. A., and Klee, W.A.: Deoxymorphines: roles of the phenolic hydroxyl in antinociception and opiate receptor interactions. J. Med. Chem. 22: 256-259, 1979.

Jacobson, A.E., Rice, K.C., Reden, J., Lupinacci, L., Brossi, A., Streaty, R.A., and Klee, W.A.: Paradoxical effects of N-cyanoalkyl substituents upon the activities of several classes of opioids. J. Med. Chem. 22: 328-331, 1979.

Klee, W.A.: Dual regulation of adenylate cyclase: a biochemical model for opiate tolerance and dependence. In Fishman, J. (Ed.): The Bases of Addiction. Berlin, Dahlem Workshops, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00935-12 LGCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. S. Adhya	Geneticist	LMB	NCI
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	L. Lipkin	Head, Image Processing Unit	LP	NCI
	P. Lempkin	Computer Scientist	LP	NCI
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	R. C. Switzer	Research Fellow	LBED	NIMH
	G. C. Salmiraghi	Associate Director for Research		NIAAA

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Laboratory of Clinical Science, NIMH

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section of Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two-dimensional electrophoresis techniques were refined to study complex cell lysates. By comparison of isogenic strains of *E. coli* containing mutations in each of the enzymes of the galactose operon, it has been possible to locate the position of each of these enzymes in the two-dimensional electrophoretograms. The position of the kinase enzyme has been corraborated by electrophoresis of double antibody precipitates of the enzyme. Knowledge of the location of these enzymes will facilitate studies of gene expression of lambda transducing virus, carrying the galactose operon. A number of host proteins have been found to be activated by induction of lambda virus, when it is integrated in the host chromosome. These two-dimensional electrophoresis techniques have been used to screen for biochemical abnormalities in human genetic diseases. This effort has been augmented by the development of computer techniques to analyze the complex patterns obtained from body fluids and cell lysates. An alkaline polypeptide, of apparent molecular weight 15,000 daltons, has been observed in the urine of patients with familial Alzheimer's disease, a similar polypeptide has not yet been found in controls. A highly sensitive protein stain has been developed which is 100-times more sensitive than the previous used Commassie Blue stain.

Project Description:

The enzymes of the galactose pathways have been studied by two-dimensional electrophoresis. By electrophoresing cell lysates of isogenic strains of *E. coli* containing mutations in the galactose operon enzymes, the positions of the enzymes in the two-dimensional patterns could be deduced. The localization of these proteins was facilitated by development of computer image processing techniques in collaboration with Drs. L. Lipkin and P. Lempkin. The enzyme locations have been varified by the use of enzyme specific double-antibody precipitates. Induction of the prokaryotic virus, lambda in *E. coli*, has been shown to result in changes in host protein synthetic patterns which were unsuspected prior to the application of two-dimensional electrophoretic techniques to the complex whole cell lysates. In a separate study, RNA chain termination was demonstrated to be due in some cases to a direct interaction between the host protein rho and RNA polymerase.

Applications of the two-dimensional electrophoretic techniques as a screen for biochemical abnormalities in various genetic diseases was hindered by the lack of sensitivity for staining trace proteins. In collaboration with Dr. R. Switzer, a highly sensitive silver stain has been developed which allowed the detection of as little as 0.1 ng/mm^2 of protein in polyacrylamide gels. This stain permits detection of proteins in unconcentrated body fluids such as spinal fluid, amniotic fluid and urine, and also allows detection of trace proteins in cell lysates, which hitherto required radioactive labelling and autoradiography for detection.

Analysis of clinical material from patients with degenerative diseases of the central nervous system has revealed a low molecular weight alkaline polypeptide in the urine of patients with familial Alzheimer's disease. A similar polypeptide has not yet been found in other diseases, such as Huntington's disease or in the normal urines.

Publications:

Merril, C.R., Leavitt, J., Van Keuren, M.L., Ebert, M.H., and Caine, E.D.: Hypoxanthine guanine phosphoribosyltransferase (HGPRT) in Gilles de la Tourette syndrome. Neurology 29: 131-134, 1979.

Geier, M.R., Stanbro, H., and Merril, C.R.: Endotoxins in commercial vaccines. Appl. Envir. Microbiol. 36: 445-449, 1978.

Das, A., Merril, C.R., and Adhya, S.: Interaction of RNA polymerase and rho in transcription termination: couples ATPase. Proc. Nat. Acad. Sci. U. S. A. 75: 4828-4832, 1978.

Switzer, R.C., III, Merril, C.R., and Shifrin, S.: A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anal. Biochem., in press.

Merril, C.R., Switzer, R.C., and Van Keuren, M.L.: Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. Nat. Acad. Sci. U. S. A., in press.

Lempkin, P., Merril, C., Lipkin, L., Van Keuren, M., Oretel, W., Shapiro, B., Wade, M., Schultz, M., and Smithe, E.: Software aids for the analysis of 2-D gel electrophoresis images. Computers Biomed. Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00936-16 LGCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Homocystinuria: Methionine Metabolism in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB	NIMH
OTHER:	R. Havlik	Medical Officer, Epidemiology Branch	HV	NHLBI
	M. Feinleib	Chief, Epidemiology Branch	HV	NHLBI

COOPERATING UNITS (if any)

Division of Heart and Vascular Diseases, NHLBI

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/12	4/12	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A survey of the health status of parents and grandparents of cystathionine synthase deficient patients is being continued. The aim is to determine whether heterozygotes for this genetic defect are at increased risk for coronary artery disease. By appropriate physician contact we have now been able to obtain responses from more than 190 families with homocystinuric patients, 113 with new-mutation achondroplastic dwarfism, 125 with hyperphenylalaninemic, and 35 with cystinotic children. Most of the information has been entered in a computer and a preliminary analysis carried out. This preliminary analysis suggests that the questionnaire approach has yielded valid data since the findings in the control groups are reasonable in comparison with data obtained in more elaborate epidemiological studies. It appears, also, that interpretation of the data will be made more rigorous by (a) addition of further control families (b) documentation of the ages at which initial coronary attacks occurred in fathers who have had non-fatal attacks of this kind, and (c) obtaining approximate years of birth and death for grandparents for whom such dates are not known exactly. Efforts to obtain such information are now underway.

Project Description:

During the interval since the last annual report, most of our efforts on this project have been devoted to the epidemiological study which seeks to determine whether heterozygotes for cystathionine synthase deficiency are at increased risk for early coronary artery disease. If, as is suggested by certain data in the literature previously reviewed here, these heterozygotes are at greatly increased risk, one would provide an explanation for the major portion of early coronary artery disease not presently associated with known risk factors. Further, assessment of such risk may help to evaluate the view-point, currently receiving attention in the semi-popular press (see, for example, "Beyond Cholesterol. A New Theory on Arteriosclerosis" by E.R. Gruberg and S.A. Raymond, Atlantic, May 1979) which suggests that homocystinemia may be a major factor in the causation of arteriosclerosis in the United States.

Considerable progress has been made on this project. Through the efforts of more than 100 physicians we have now obtained suitably completed questionnaires from 192 families with homocystinuric (cystathionine synthase deficiency type) children. Of these, 125 were from North America (U.S. and Canada), the remainder from England, Scotland, Northern Ireland, Eire, Australia and New Zealand. Since 255 families were contacted, this represents an overall response rate of more than 75%. The families contacted almost surely are a reasonably complete sampling of the known families of this type in the English-speaking world, and it is expected that this group can be expanded little, if at all, in the near future.

Among the control groups, responses have been obtained from 113 families with new-mutation achondroplastic dwarfism (78% of those contacted), 125 families with hyperphenylalaninemia (including classical PKU), and 35 with cystinosis. Efforts are now underway to expand the two former groups to the region of 140-150 responses from families with dwarfism and 150-160 families with hyperphenylalaninemia. Cystinosis families are too limited for much expansion.

The available data have been entered into a computer, and programs have been developed for suitable analysis. Because data collection is continuing, any such analysis at this time must be regarded as preliminary. However, the results do indicate that the questionnaire approach adopted for this survey yields data for the control groups which are reasonable in the light of knowledge gained from other epidemiological studies of more elaborate design. For example, these groups reveal the expected sex-dependent differences in coronary attacks between fathers and mothers, or between grandfathers and grandmothers, and there are reasonable age-dependent increases in the incidence of fatal coronary attacks for each group studied. These results are encouraging in validating the method and offer assurance that the data, when obtained in final form, will be reliable.

The preliminary analysis has also pinpointed certain areas in which the data are as yet incomplete. Most important are: (a) Knowledge of the age at which fathers who have had a non-fatal heart attack had their first such attack. This information was not elicited on the standard forms to avoid undue emphasis on questions about coronary artery disease. Availability of these data would permit construction of a complete table for fathers (the group of most interest) of incidence of coronary artery attacks, both fatal and non-fatal, as functions of age. (b) Approximations of the years of birth (and death) of grandparents for those for whom the exact year is unknown. Such approximations would permit inclusion in the results of a considerable additional number of grandparents (as many as 25% of the present samples), thereby minimizing the chance that any bias has inadvertently been introduced through omission of this group because of incomplete data. We are now in the process of attempting to obtain this additional information by follow-up questionnaires.

It is expected that virtually all of the data will have been collected for this survey within the next 3-4 months. Because analytical methods have been developed, further data analysis should be completed soon thereafter. Hopefully, a reliable assessment of the impact of a moderate degree of homocystinemia, both for the individual concerned, and as a public health problem, will then be available.

Publications:

Schulman, J.D., Agarwal, B., and Mudd, S.H., (letter): Pulmonary embolism in a homocystinuric patient during treatment with dipyridamole and acetylsalicylic acid. N. Eng. J. Med. 299: 661, 1978.

Mudd, S.H., Ebert, M.H., and Scriver, C.R.: Labile methyl group balances in the human: the role of sarcosine. Metabolism, in press, 1979.

Mudd, S.H.: Diseases of sulphur metabolism: implications for the methionine-homocysteine cycle, and vitamin responsiveness. Ciba Foundation Symposium 72: Biology of Sulphur, in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00937-14 LGCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Transsulfuration in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Giovanelli	Research Chemist	LGCB NIMH
	A. H. Datko	Botanist	LGCB NIMH
	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2 8/12	PROFESSIONAL: 2 8/12	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The following aspects of the growth and physiology of the higher plant *Lemna* have been clarified: (a) Three stages in the vegetative reproduction of *Lemna* were defined. Although all cultures contain colonies in these three stages of development, it was demonstrated that these cultures are essentially equivalent in terms of specific rates of net synthesis of methionine and other amino acids. (b) A growth medium for *Lemna* was developed which is as good (at high sulfate concentrations) or better (at low sulfate concentrations) than the most commonly used medium. (c) In the presence of the sulfate analogs molybdate or selenate, *Lemna* colonies have been maintained under conditions of sulfur limitation and with stable growth rates and protein contents for as long as 18 doublings. These analogs promise to be useful tools in our work. In *Lemna* we have previously demonstrated feedback regulation by methionine of *de novo* synthesis of its sulfur moiety, probably at the cystathionine synthesis step. Preliminary results suggest that methionine also feedback regulates *de novo* synthesis of its C4 moiety at the same locus. *De novo* synthesis of the methyl group tentatively appears to be regulated less than that of the C4 moiety of methionine.

Project Description:

The higher plant Lemna has many advantages for studies on the mechanism of control of methionine biosynthesis, and we plan to continue to use this plant for future experiments. However, in spite of the popularity of Lemna as an experimental material, many aspects of its growth and physiology are not clear. We have continued our studies (summarized below) on the growth and physiology of Lemna, since a better understanding of certain of these processes would be of value in the design and interpretation of our experiments:

(a) Lemna cultures are composed of colonies at different stages of development, and it is not known to what extent these different stages can affect the physiology of the culture, especially with respect to the synthesis of methionine. Three individual stages in the vegetative reproduction of Lemna were defined, each with a characteristic doubling time. Type A colonies are newly separated daughters producing their first daughters (doubling time of 60 hrs). Type B colonies are producing their second or subsequent even-numbered daughters (doubling time of 30 hrs). Type C colonies are producing their third or subsequent odd-numbered daughters (doubling time of 40 hrs). It was shown with a mathematical model that, irrespective of the type of colony used for inoculation, a culture approaches an equilibrium state containing a fixed proportion (Types A:B:C = 0.6: 0:22: 0.18) of each type of colony. The relative proportions of each type of colony found in cultures chosen at random over a period of 6 months agreed closely with those predicted from the model. All three types of colonies showed an essentially constant specific rate of net protein synthesis. Therefore, individual cultures of Lemna may be considered essentially equivalent in terms of their specific rates of net synthesis of methionine and other amino acids, because the three types of colonies have essentially the same specific rates of net protein synthesis, and because individual cultures approach the same equilibrium composition of the three types of colonies.

(b) We have developed a growth medium for Lemna which is as good (at high sulfate concentrations) or better (at low sulfate concentrations) than the commonly used medium.

(c) Growth of Lemna in the presence of the sulfate analogs molybdate or selenate (at low concentrations of sulfate) results in symptoms of sulfur limitation (e.g. multifronded colonies, reduction in growth rate and protein content) that can be relieved by addition of higher concentrations of sulfate. Lemna colonies have been maintained with stable growth rates and protein content for as long as 18 doublings in the presence of each analog. Thus molybdate and selenate, which appear to interfere with sulfur metabolism in plants by different mechanisms, promise to be useful tools in our work. For example it may be possible to use molybdate to grow Lemna under constant conditions of sulfur limitation, which could be accomplished previously only in a chemostat type of apparatus.

A major goal of our research is to elucidate the mechanism of control of the biosynthesis of methionine and the other amino acids (lysine, threonine and isoleucine) of the aspartate family. The first three amino acids are the "first-limiting" constituents in almost all protein-poor diets. Clearly, an understanding of the regulatory patterns for synthesis of these amino acids would be important in any rational approach to provision of better nutrition worldwide. We have previously demonstrated that growth of Lemna in the presence of exogenous methionine results in feedback regulation of the de novo synthesis of the sulfur moiety of this amino acid, probably at the cystathionine synthesis step. This work has now been extended to determine whether methionine also regulates de novo synthesis of its C4 and methyl moieties. In these experiments Lemna was grown on sucrose-C^{14} as a sole source of carbon, and the effect of exogenous methionine on the incorporation of C^{14} into cystathionine and its products (homocysteine, methionine, AdoMet, AdoHcy, etc) determined. Special methods were developed for isolation of the relatively small amounts of methionine-C^{14}, and for determination of the distribution of radioactivity in the C4 and methyl moieties of this amino acid. Our analyses are not yet complete, but preliminary indications are that de novo synthesis of the C4 moiety of methionine is inhibited by exogenous methionine; de novo synthesis of the methyl group tentatively appears to be inhibited less than that of the C4 moiety. By contrast, de novo synthesis of threonine is not appreciably inhibited by growth in the presence of exogenous methionine. These findings are consistent with feedback regulation of the C4 moiety of methionine at the cystathionine synthesis step.

A significant effort was devoted to preparing a comprehensive review on sulfur amino acids in plants, which we were invited to submit for publication in a multi-volume series entitled, "Biochemistry of Plants - A Comprehensive Treatise".

Future work will be continued along the following lines:

(1) Our combined evidence with Lemna strongly suggests that the locus of control for methionine biosynthesis in plants is at the cystathionine synthase step. Two mechanisms for control at this step have been proposed. In the first, methionine (or AdoMet) directly regulates the activity of cystathionine synthase. In the second, AdoMet stimulates threonine synthase, thereby decreasing the concentration of O-phosphohomoserine, and consequently the rate of cystathionine synthesis. As a first step in establishing which of these mechanisms is of physiological significance in plants, it will be determined whether threonine synthase of Lemna is stimulated by AdoMet. If it is, the physiological significance of the two mechanisms will be distinguished by determining the effects of feedback inhibition by methionine on the concentration of O-phosphohomoserine in Lemna - the first mechanism requires that the concentration of O-phosphohomoserine increase during feedback inhibition by methionine, while the second requires that the concentration of O-phosphohomoserine decrease. Sensitive and specific methods that have been developed in this laboratory will be used to assay O-phospho-

homoserine.

(2) Despite its strategic position in the control of methionine biosynthesis in plants, the properties of plant cystathionine γ-synthase have not been systematically examined. It is proposed to purify this physiologically important enzyme from Lemna, and to study its catalytic and regulatory properties. Of interest is whether direct sulfhydration to form homocysteine is also catalyzed by cystathionine γ-synthase (as in bacteria), or whether a separate enzyme is involved (as in fungi).

Publications:

Giovanelli, J., Mudd, S. H., and Datko, A. H.: Homocysteine biosynthesis in green plants. Physiological importance of the transsulfuration pathway in *Chlorella sorokiniana* growing under steady-state conditions with limiting sulfate. *J. Biol. Chem.* 253: 5665-5667, 1978.

Datko, A. H., Mudd, S. H., Macnicol, P. K., and Giovanelli, J.: A phytostat for the growth of *Lemna* in semicontinuous culture with low sulfate. *Plant Physiol.* 62: 622-628, 1978.

Datko, A. H., Mudd, S. H., Giovanelli, J., and Macnicol, P. K.: Sulfur containing compounds in *Lemna perpusilla* 6746 grown at a range of sulfate concentrations. *Plant Physiol.* 62: 629-635, 1978.

Giovanelli, J., Mudd, S. H., and Datko, A. H.: Sulfur amino acids in plants. In Stumpf, P. K., and Conn, E. E. (Eds.): *Biochemistry of Plants*, Volume 5, Chapter 12. New York, Academic Press, in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH-00981-14-LNB

PERIOD COVERED
July 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Analysis of the Macromolecular Structure of Nerve Membrane During Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Tasaki	Chief	LNB NIMH
	S. Terakawa	Visiting Associate	LNB NIMH
	R. Gibbons	Electronic Tech.	LNB NIMH

COOPERATING UNITS (if any)
Marine Biological Laboratory
Woods Hole, Mass.

LAB/BRANCH
Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION
NIMH: ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.8	1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of nerve excitation was studied from a macromolecular viewpoint. The miniature responses of squid giant axons were analyzed using a real-time spectrum-analyzer. These responses were found to be sensitive both to tetrodotoxin and to tetraethylammonium ions. This finding seriously challenges the dual-channel hypothesis and presents strong evidence suggesting the existence of only one kind of ion channel in nerve membrane. Another major advance in understanding the mechanism of nerve excitation was made when we discovered that a squid giant axon could remain excitable when perfused both intracellularly and extracellularly with an identical solution containing only a divalent salt such as the salts of cobalt, manganese, nickel or barium. This finding reduces the number of ions involved in the excitation process sufficiently to allow the physico-chemical theory of excitation to be advanced.

PHS-6040
(Rev. 10-76)

The present studies of the periodic miniature responses have led Dr. I. Tasaki to a new interpretation of the origin of "periodicity" in the activity of the nervous system. The results of these new studies will be presented in the near future both in a monograph entitled "Physiology and Electrochemistry of Nerve Fibers" and in an invited manuscript which will be published in Physiological Reviews.

PUBLICATIONS

Baumgold, J., Matsumoto, G. and Tasaki, I.: Biochemical studies of nerve excitability: The use of protein modifying reagents for characterizing sites involved in nerve excitation. J. Neurochem. 30: 91-100, 1978.

Matsumoto, G., Tasaki, I. and Inoue, E.: Oscillatory subthreshold responses and potential fluctuation observed in squid giant axons. J. Phys. Soc. Japan 44: 351-352, 1978.

Metuzals, J. and Tasaki, I.: Subaxolemmal filamentous network in the giant nerve fiber of the squid (Loligo pealei L.) and its possible role in excitability. J. Cell Biol. 78: 597-621, 1978.

Tasaki, I.: Chemical and electrical receptors on the surface of squid giant axons. Brain Res. Bull. 4(1): 190-191, 1979.

Tasaki, I.: Chemical stimulants and real-time spectrum analyzer used for studying properties of membrane excitable sites. In Chalazonitis, N. and Boisson, M. (Eds.): Abnormal Neuronal Discharges. New York, Raven Press, 1978, pp. 233-242.

Tasaki, I.: Further studies of periodic miniature responses in squid giant axons. Jap. J. Physiol. 28: 89-108, 1978.

Tasaki, I. and Matsumoto, G.: Optical probes of axonal membrane. In Waxman, S.G. (Ed.): Physiology and Pathobiology. New York, Raven Press, 1978, pp. 237-250.

Yoshioka, T., Pant, H.C., Tasaki, I., Baumgold, J., Matsumoto, G. and Gainer, H.: An approach to the study of intracellular proteins related to the excitability of the squid giant axon. Biochim. Biophys. Acta. 538: 616-626, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00982-11-LNB

PERIOD COVERED

July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Sensory-motor Integration in the Primate Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.H. Wurtz	Research Physiologist	LNB NIMH
	B.J. Richmond	Staff Associate	LNB NIMH
	S.J. Judge	Visiting Fellow	LNB NIMH
	J.E. Albano	Guest Worker	LNB NIMH
	H.C. Healy	Psychology Technician	LNB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH:ADAMHA: NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal investigator has transferred to the Eye Institute and the project is terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00983-1-LNB

PERIOD COVERED

July 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on the Mechanism of Nerve Conduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

H. Pant	Senior Staff Fellow	LNB NIMH
J. Baumgold	Senior Staff Fellow	LNB NIMH

COOPERATING UNITS (if any)

Marine Biological Laboratory
Woods Hole, Mass.

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH: ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Proteins involved in nerve excitation and conduction were studied using a variety of biochemical approaches. The most direct approach involved the use of radioactively labeled tetrodotoxin and saxitoxin. These toxins bind specifically to proteins involved in nerve excitation and are thus a very convenient probe for studying these proteins. The involvement of lipids and fatty acids in this binding was studied and found to be important in stabilizing the binding sites. Another approach involved studying the proteins from the interior of the squid giant axons. This approach revealed the presence of a calcium-activated protease in the axoplasm of this cell. The substrate for this protease was found to be neurofilament protein, a protein that may well be involved in the process of nerve conduction. Since calcium accumulates inside an axon during excitation, this work has some profound implications regarding nerve conduction, implications which will be studied during the forthcoming year. The binding of radioactive calcium to membrane fragments isolated from crab nerve was also studied. This binding was found to be dependent on the monovalent cation species in the buffer.

PUBLICATIONS

Pant. H.C., Terakawa, S. and Gainer, H.: A calcium activated protease in squid axoplasm. *J. Neurochem*. 32: 99-102, 1979.

Pant, H.C., Shecket, G., Gainer, H. and Lasek, R.J.: Neurofilament protein is phosphorylated in the squid giant axon. *J. Cell Biology* 78: R23-27, 1978.

Pant, H.C., Yoshioka, T., Tasaki, I., and Gainer, H.: Divalent cation dependent phosphorylation of proteins in squid giant axon. *Brain Res*. 162: 303-313, 1979.

Pant, H.C., Terakawa, S., Baumgold, J., Tasaki, I. and Gainer, H.: Protein release from the internal surface of the squid giant axon membrane during excitation and potassium depolarization. *Biochim. Biophys. Acta* 513: 132-140, 1978.

Pant, H.C., Terakawa, S., Yoshioka, T., Tasaki, I., and Gainer, H.: Evidence for the utilization of extracellular [γ32P]ATP for the phosphorylation of intracellular proteins in the squid giant axon. *Biochim. Biophys. Acta*. 582: 107-114, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01031-11 LNC

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Conversion of Phenylalanine to Tyrosine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Sheldon Milstien	Research Chemist	LNC NIMH
	Michael Parniak	Visiting Fellow	LNC NIMH
	Lauraine Shaw-Goldstein	Staff Fellow	LNC NIMH
	Hiroyuki Hasegawa	Visiting Fellow	LNC NIMH
	Harvey Wilgus	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda,Maryland 20205

TOTAL MANYEARS: 6.5	PROFESSIONAL: 5.5	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two new procedures have been developed that permit the early diagnosis of hyperphenylalaninemia due to a deficiency of biopterin and due to a deficiency of dihydropteridine reductase.

Triaminohydroxy pyrimidine has been found to have cofactor activity with purified phenylalanine hydroxylase.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this research project is the detailed description of the complex enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components of the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase, and tetrahydrobiopterin.

A related objective is to use this basic knowledge of the phenylalanine hydroxylase system to increase our understanding of the disease, phenylketonuria, (PKU), as well as its variants. Specifically, the goal is to devise methods of detection and therapy for these diseases, as well as to understand their pathogenesis.

During the last year, we have succeeded in developing two new tests that permit the early detection of the two different forms of phenylketonuria (PKU) or hyperphenylalaninemia (HPA) that are caused by defects in dihydropteridine reductase (DHPR) and in biopterin biosynthesis.

The new test for DHPR deficiency is based on our observation that when normal urine is collected in the presence of a reducing agent, most of the biopterin is present as the tetrahydro form. In contrast, urine from a DHPR-deficient patient contains essentially no tetrahydrobiopterin. The absence of tetrahydrobiopterin in urine, therefore, is diagnostic for DHPR-deficiency.

The new test for HPA due to a block in the *de novo* synthesis of biopterin is based on the finding that patients with this disease, who have low urinary levels of biopterin, excrete large amounts of neopterin. Neopterin has long been suspected to be a precursor of biopterin. Neopterin and biopterin are separated and quantitatively measured by high-pressure liquid chromatography. Our findings, therefore, provide strong support for the precursor-product relationship of these two pterins.

We have continued to study the *in vivo* regulation of phenylalanine hydroxylase, concentrating on the way in which the enzyme's activity is controlled by phosphorylation-dephosphorylation processes. We have found that administration to rats of theophylline, a drug that is known to elevate levels of cyclic-AMP, and by that mechanism to increase the degree of phosphorylation of susceptible enzymes, activates phenylalanine hydroxylase. The activation and the change in some of the properties of the enzyme indicate that its' state of phosphorylation increases under the influence of the drug. Since this drug is already being used in other clinical conditions, we are planning to administer it to PKU variants who have residual phenylalanine hydroxylase activity to determine if that activity can be increased in these patients.

In other studies with phenylalanine hydroxylase, we have found that 2,4,5-triamino-6-hydroxypyrimidine, a relatively simple analogue of the cofactor, tetrahydropterin, has cofactor activity with phenylalanine hydroxylase. Furthermore, a product of the pyrimidine is a substrate for DHPR. This is the first example of a non-pterin compound having cofactor activity in the phenylalanine hydroxylase system. Since the pyrimidines are considerably more stable than tetrahydropterins, this finding should facilitate studies on the mechanism of action of phenylalanine hydroxylase.

Publications:

Kaufman, S., Berlow, S., Summer, G.K., Milstien, S., Schulman, J.D., Orloff, S., Spielberg, S. and Pueschel, S.: Hyperphenylalaninemia due to a deficiency of biopterin. A variant form of phenylketonuria. New Eng. J. Med. 299: 673-679, 1978.

Grobe, H., Bartholome, K., Milstien, S. and Kaufman, S.: Hyperphenylalaninemia due to dihydropteridine reductase deficiency. Eur. J. Pediatr. 129: 93-98, 1978.

Donlon, J. and Kaufman, S.: Glucagon stimulation of rat hepatic phenylalanine hydroxylase through phosphorylation *in vivo*. J. Biol. Chem. 253: 6657-6659,

Kaufman, S.: The enzymes of the hepatic phenylalanine hydroxylating system. J. Inher. Metab. Dis. 1: 63-65, 1978.

Chang, N., Kaufman, S. and Milstien, S.: The mechanism of the irreversible inhibition of rat liver phenylalanine hydroxylase due to treatment with p-chlorophenylalanine. J. Biol. Chem. 254: 2665-2668, 1979.

Brewster, T.G., Moskowitz, M.A., Kaufman, S., Breslow, J.L., Milstien, S. and Abroms, I.F.: Dihydropteridine reductase deficiency associated with severe neurologic disease and mild hyperphenylalaninemia. Pediatrics 63: 94-99, 1979.

Kaufman, S.: Biopterin and metabolic disease. In Kisliuk, R.K. and Brown, G.M. (Eds.): Chemistry and Biology of Pteridines. New York, Elsevier, 1979, pp. 117-124.

Kaufman, S.: The activity of 2,4,5-triamino-6-hydroxypyrimidine in the phenylalanine hydroxylase system. J. Biol. Chem. 254: 5150-5154, 1979.

Kaufman, S. and Shaw-Goldstein, L.: Oxidation-reduction reactions of 2,4,5-triamino-6-hydroxypyrimidine and its cofactor activity in the phenylalanine hydroxylase system. Proceedings of the Third International Symposium on Oxidases and Related Redox Systems (ISOX), in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01032-11 LNC

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Robert J. Pollock	Senior Staff Fellow	LNC NIMH
	Gregory Kapatos	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synthesis-secretion coupling in catecholamine-containing cells is believed to occur via modulation of the rate-limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase. We have demonstrated that tyrosine hydroxylase is activated by phosphorylating conditions and that the kinetic expression of this activation is dependent upon pH. These alterations in tyrosine hydroxylase activity may supply a mechanism for synthesis-secretion coupling.

PHS-6040
(Rev. 10-76)

Project Description:

Cells which are specialized to secrete catecholamines couple biosynthetic and secretory processes so as to maintain steady-state levels of catecholamine despite alterations in secretory rate. Synthesis-secretion coupling is believed to occur via modulation of the rate limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase, by some mechanism linked to the secretory process.

We have previously shown that when tyrosine hydroxylase is incubated under phosphorylating conditions the kinetic properties of the enzyme are altered. The Km for the tetrahydropterin cofactor is decreased, the Ki for catecholamines is increased; and the pH optimum is shifted to higher pH. These alterations would result in an increase in tyrosine hydroxylase activity under conditions of accelerated catecholamine release in which the tetrahydropterin presumably is not saturating, catecholamines are present and the pH is near neutrality.

However, recent reports have demonstrated that the activation of tyrosine hydroxylase by phosphorylating conditions is expressed not as a decrease in Km but rather as an increase in V max. In contrast to the expression of a Km alteration, such a V max change would imply regulation independent of reduced pterin levels.

Utilizing a partially purified preparation of bovine caudate tyrosine hydroxylase, a detailed analysis of kinetic alterations in response to phosphorylating conditions was undertaken at pH values ranging from 5.7 to 7.2.

Under control conditions both the Km for cofactor and V max declined as pH was increased. Following treatment under phosphorylating conditions, Km and V max declined at pH 5.7; and no activation was therefore evident. At pH 6.2 activation was expressed only as a decrease in Km, while at pH 7.2 the major component of activation was an increase in V max. These pH-dependent effects were observed with both synthetic and the natural pterin cofactor, and a chemical or enzymatic cofactor regenerating system.

A similar analysis was performed with the use of rat striatal synaptosomes. Tyrosine hydroxylase was activated _in situ_ by phosphorylating conditions produced by incubation of synaptosomes with the cAMP analogue, 8 Bromo cAMP. This treatment was combined with the addition to the incubation medium of various concentrations of reduced tetrahydropterin.

The results of this experiment demonstrated that at pH 7.2 activation is expressed primarily as an increase in V max, although a decrease of smaller magnitude in what could be interpreted as Km was also observed. These data therefore support our observations made with partially purified tyrosine hydroxylase.

The significance of these observations are: first, at the physiological pH of 7.2 the Km of tyrosine hydroxylase for cofactor approached a value near the estimated concentration of cofactor *in vivo*. The apparent disparity between these values has led some to question whether tetrahydrobiopterin is the natural cofactor. Second, alterations in the environmental pH of tyrosine hydroxylase can be of regulatory significance. At pH 7.2 the expression of activation is relatively independent of cofactor concentration while at more acidic conditions this is not the case.

Publications:

Hoeldtke, R., and Kaufman, S.: A tritium-release *in vivo* assay of dopamine-β-hydroxylase activity in sympathetic neurons. *Biochem. Pharmacol*. 27: 2499-2506, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01034-11 LNC

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Biochemical Basis of Skeletal Muscle Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Herman Vandenburgh	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

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Laboratory of Neurochemistry

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include the relationship between stretch-induced hypertrophy and muscle protein synthesis, degradation, and changes in Na pump activity and cyclic nucleotide levels.

PHS-6040
(Rev. 10-76)

Project Description:

One hypothesis of the basis of skeletal muscle hypertrophy is that it is due to mechanical stretching of the muscle which leads to increased tension and increased work. This hypothesis has been confirmed using a technique developed in this laboratory of stretching monolayers of fowl embryonic skeletal myotubes differentiated *in vitro*. A continuous 10 percent stretch of the cells leads to increased amino acid accumulation, increased incorporation of amino acids into total cellular protein and muscle specific protein (myosin heavy chain), increased accumulation of total cellular protein and myosin heavy chain and decreased total cellular protein degradation. These changes are similar to those seen in *in vivo* models of skeletal muscle hypertrophy. We have automated the stretching procedure and are currently looking at the effect of multiple stretches, and of stretch and relaxation, on these hypertrophy factors. Having established an *in vitro* model for skeletal muscle hypertrophy, we are currently testing several biochemical models for the stimulation of cell growth in this system. These models include changes in the activity of the sodium pump and in intracellular levels of cyclic nucleotides.

Publications:

Vandenburgh, H.H. and Kaufman, S.: *In vitro* model for stretch-induced hypertrophy of skeletal muscle. *Science* 203: 265-268, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01035-11 LNC

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Process of Lysogeny

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Nash	Medical Officer (Research)	LNC NIMH
OTHER:	Yoshiko Kikuchi	Visiting Associate	LNC NIMH
	Thomas Pollock	Guest Worker	LNC NIMH
	Carol Robertson	Biologist	LNC NIMH

COOPERATING UNITS (if any) Division of Biology and Medicine, Brown University; Laboratory of Molecular Genetics, NICHD; Division of Cancer Biology, Frederic Cancer Research Center; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD; Department of Microbiology, Univ. of Michigan Medical School.

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The components of a naturally-occurring genetic recombination between viral and host DNA have been defined and purified. One of the proteins required for this reaction, the bacteriophage lambda Int protein, has been shown to behave as a topoisomerase, i.e. to break and reseal the strands of DNA so as to relax supertwisted DNA. This is the first case in which a topoisomerase has been directly implicated in genetic recombination and provides new insight into the function of these ubiquitous proteins. Int protein also binds specifically to DNA, covering two closely spaced sequences one of which is centered at the site of the genetic recombination. Other studies of site specific recombination have uncovered unusual conditions that permit relaxed DNA to function as the substrate for this reaction, clearing the way for comparative studies to determine the role of DNA supercoiling in a biological reaction.

PHS-6040
(Rev. 10-76)

Project Description:

Rearrangement of DNA is thought to be a major mechanism in the genesis of variation between individuals, in the differentiation of specialized functions within the tissues and organs of an individual, and in the response of the genetic apparatus to environmental stimuli. This laboratory has been studying the biology and biochemistry of a genetic rearrangement that, in nature, inserts the DNA of a virus into the chromosome of its bacterial host. Our study, the first to characterize a naturally-occurring genetic recombination in biochemical detail, has defined the essential components of this reaction. They are: (a) supercoiled DNA bearing the specific DNA sequences (attachment sites) at which the recombination takes place, (b) a viral protein, the product of the bacterial phage int gene, (c) a bacterial protein that appears to involve the participation of two E. coli genes, and (d) the polyamine spermidine. Our current efforts are centered on determining how these various components, all of which have been extensively purified for the first time in this laboratory, act separately and together to produce recombined DNA.

Our experiments of the past year have uncovered two major new findings concerning the mechanism of action of Int protein. First, we have discovered that Int protein is a topoisomerase - i.e. that Int acting alone can break and reseal the strands of DNA. We have used both genetic and biochemical criteria to show that Int protein itself, and not a contaminant of our purified preparation, has this activity. The topoisomerase activity of Int is remarkably like that isolated from the nuclei of human and other animal cells. This suggests that these latter enzymes, for which no function has been discerned, may be involved in genetic recombination in eukaryotes. Consideration of the role of the topoisomerase activity of Int in integrative recombination has led us to propose a novel mechanism for the exchange of DNA strands. Experimental tests of this mechanism are currently under design. The second major finding concerning Int protein is our determination, in collaboration with the laboratory of Dr. A. Landy of Brown University, of DNA sequences that interact with Int protein. Our earlier studies had proven that Int interacts specifically with DNA from the attachment site region and that the complex is sufficiently tight to protect some DNA from digestion by exogenous nucleases. The present study has located these protected sequences. Suprisingly, we find that two separate sequences, each 30-35 nucleotide pairs long and separated by 60 nucleotide pairs, interact with Int. The two sequences have very different primary structures suggesting that Int protein may encompass two binding sites, one for each of the two distinct subregions of the attachment site. One of these regions includes the locus of the recombination crossover, the other region may be involved in the preliminary positioning of Int protein at the correct location of DNA. We will extend these studies to determine the effect of the other components of the recombination reaction on the interaction of Int and DNA.

Our previous studies have shown that efficient recombination in vivo and in vitro requires a supercoiled DNA substrate. Recently, we have discovered that when reactions are carried out at low ionic strength, nonsupercoiled (relaxed) DNA can be recombined. We have characterized this reaction and find that like supercoiled DNA, relaxed DNA requires spermidine plus Int and bacterial protein. Moreover, recombination with relaxed DNA goes to completion, producing a pair of rearranged sequences with no breaks or gaps in the DNA structure. However, even under optimal conditions, relaxed DNA recombines very slowly, at one-tenth to one-fiftieth of the rate of supercoiled DNA substrate. Although the physiological significance of recombination with relaxed DNA is in doubt, we believe it offers a unique opportunity for comparative studies to determine the role of supercoiling in a biological reaction.

Recombination between pairs of DNA molecules that share extensive regions of homology or identity is a feature of virtually all living systems. Superficially, such homologous recombination contrasts with the site specific recombination studied in this laboratory, in that the latter recombines essentially nonhomologous DNA only at a very short identical sequence. We have analyzed recombination in vivo to ask whether the two kinds of recombination might share a similar mechanism. Briefly, we have shown that the int gene can act to promote crossover events at attachment sites that result in recombination of distant genes. This implies that Int promoted recombination produces as an intermediate a partially recombined structure in which the two parents are held together by a mobile joint. This mobile joint, consisting of a region where one DNA strand from each parent has broken and rejoined to a new partner, has long been known as an intermediate in homologous recombination. We therefore conclude that site specific and homologous recombination proceed by related mechanisms. In collaboration with Dr. R. Weisberg of NICHD, we are currently developing a new method for the detection and isolation of such joints and will pursue their formation during recombination in vitro.

Publications:

Mizuuchi, K., Gellert, M., Nash, H.A.: Involvement of supertwisted DNA in integrative recombination of bacteriophage lambda. J. Mol. Biol. 121: 375-392, 1978.

Kikuchi, Y, and Nash, H.A.: The bacteriophage λ int gene product. J. Biol. Chem. 253: 7149-7157, 1978.

Kikuchi, Y. and Nash, H.A.: Integrative recombination of bacteriophage λ: Requirement for supertwisted DNA in vivo and characterization of Int. In Cold Spring Harbor Symposia on Quantitative Biology 43: 1099-1109, 1978.

Miller, H.I., Kikuchi, A., Nash, H.A., Weisberg, R. and Friedman, D.I.: Site-specific recombination of bacteriophage λ: The role of host gene products. In Cold Spring Harbor Symposia on Quantitative Biology 43: 1121-1126, 1978.

Enquist, L.W., Nash, H. and Weisberg, R.A.: Strand exchange in site-specific recombination. Proc. Natl. Acad. Sci. 76: 1363-1367, 1979.

Kikuchi, Y. and Nash, H.A.: Nicking-closing activity associated with bacteriophage λ int gene product. Proc. Natl. Acad. Sci., in press.

Pollock, T. J. and Abremski, K.: DNA without supertwists can be an *in vitro* substrate for site-specific recombination of bacteriophage λ. *J. Mol Biol.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01036-08 LNC

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Heparin-Induced Conformations: Model for Intracellular Polysaccharide-Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Audrey L. Stone	Research Chemist	LNC NIMH

COOPERATING UNITS (if any)
New York Medical College

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Laboratory of Neurochemistry

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.85	0.55

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Physicochemical forces which sequester cell solutes from soluble form to organized structures are investigated with regard to molecular conformations of components and interacting species. Earlier results showed that ordered, negative charges (glycosaminoglycans and acidic polysaccharides) have specific ordering effects on amine cations; exhibit conformation-promoting effects on otherwise nonordered cationic polypeptides; on poly-L-lysine, all with sufficient anionic density promote a non-specific high degree of α-helical conformation. Specificity due to peptide structure revealed that heparin (and other polyanions) with poly L-lysine:L-tyrosine promotes β -protein structure, while with poly L-lysine:L-phenylalanine induces α-helical order. That the nature of conformational change in protein models due to interaction with heparin depends upon constellations of amino acids and their proclivity for α-helix or β-structures is significant in the molecular basis of multianion action in granule formation and enzyme activation. Currently, a molecular basis for heparin regulation of tyrosine hydroxylase activity, heparin in isoelectric focusing, and heparin fractionation and fractions have been studied on the basis of these molecular characterizations.

Project Description:

Anionic polysaccharides (glycosaminoglycans (GAG)) are intimately involved in the biological formation and function of inter- and intra-cellular matrices and some, such as heparin, are known to stimulate or inhibit certain enzyme systems. Intracellular storage regions, or discrete granules, occur in various organs of many species, including synaptic processes of catecholaminergic neurons. Physicochemical forces which sequester cell solutes from a soluble form to a relatively insoluble, organized structure can be investigated from the standpoint of the molecular conformations of the components and of their interacting species. Investigation of the macromolecular structure of anionic polysaccharides in solution was undertaken at the onset of this project in the early 60's with the subsequent elucidation of helical conformation in heparin and other GAG in solution.

The histology, chemical composition, and explosive release of the histamine containing granule of the mast cell were well described at that time; therefore, the heparin-histamine system was used as a conceptual model for storage and release of small potent biologically active molecules (although other multi-anion/cations apply in different systems). Thus, the conformational aspects of heparin/histamine (or cationic aromatic dyes) binding were studied using optical methods of absorption and circular dichroism (CD). The results of those early studies were the first to demonstrate ultraviolet asymmetric absorption bands for heparin and other GAG. Results with the cationic amine dyes led to the first reports of helical structures for these polysaccharides in solution. Both methods showed that GAG comprise two conformational classes in solutions near pH 7. Specific conformations were elucidated for related plant polysaccharides. Direct X-ray analysis recently performed in other laboratories now confirm the various helical patterns predicted by the early reports (M-NC-21 1964-67). The results also showed that ordered negative charges have profound and specific ordering effects on amine cations.

The current project extends these basic molecular findings to the study of a third component of inter- and intra-cellular matrices, e.g., the polypeptide. As with small amines, acid polysaccharides (PS) play a role in inducing conformational constraints in cationic polypeptides. The major objective of the current project is to quantitatively determine the conformation-altering effect of various anionic PS, and to determine possible specificity of this effect on the basis of PS conformations revealed earlier in this project. These model interactions will be applied directly to studies of enzyme activation and sequestration by heparin, and related biopolymers, in simulated matrix conditions. In this connection it is now known, from the work of Dr. Robert Rosenberg, that the anticoagulant potency of heparin, in the antithrombin system resides in less than 10 percent of the bulk preparation. Furthermore, heparin interacts with at least two lysyl residues on antithrombin so as to change its conformation and enhance its inhibition of thrombin.

The methods are measurements of molecular asymmetries by extrinsic and intrinsic Cotton effects using the Cary Models 6001-63 circular dichroism apparatus. The metachromatic reactions developed earlier in the project (measured spectrophotometrically and by circular dichroism) provide a quantitative measure of the degree of amine interactions with anionic sites. Computer models for determination of the proportion of α-helical, β- or non-ordered structure in the polypeptide reveal the degree and kind of conformation induced by a given PS. Heparin fractions were obtained by mass separation on Sephadex G-200 chromatography columns.

Studies of striatal tyrosine hydroxylase utilize dissection of mouse striatum and partial purification by means of a one-step separation by affinity chromatography.

Results using purified PS from plant, bacterial and mammalian sources, well-characterized as to the chemical nature of the anionic groups and the charge density, showed (Z01 MH 01036-04 LNC) that the non-ordered form of PLL was altered markedly by a strong electrostatic interaction with polysulfates, polyuronates, and mixed polyanionic groups on a homo- or complex polysaccharide backbone. Furthermore, there was little specificity in the interactions since both classes of GAG and all PS promoted a high degree of α-helical conformation in PLL. Subsequent studies revealed that mixed polypeptides show specificity of interaction with PS depending upon the nature of the non-basic amino acid residues. Heparin induces α-helix in L-lysine copolymer with L-phenylalanine (PLP) while copolymer L-lysine:L-tyrosine (PLT) forms the β-protein structure. Metachromatic measurements showed that the stoichiometry of the reaction was 1-to-1 with positive lysyl residues and negative heparin charges for the poly(L-lysine:L-tyrosine) and close to 1-to-1 with the poly(L-lysine:L-phenylalanine). The specificity of the biological functions of heparin and other GAG, therefore, may emerge to be due to the capacity to alter the conformations of specific amino acid sequences in a given protein, rather than to solely electrostatic interactions. This is almost certainly true for the antithrombin system. Methods for looking at the stoichiometry and interactions of certain heparin fractions have been developed which can be sensitive measurements of structure-function relationships of this potent anticoagulant biopolymer. (Some studies done in collaboration with Dr. Sigmund Lasker, New York Medical College.)

Affinity chromatographic technique for a single passage of striatal extract using heparin fixed to Sepharose 4B showed that a low degree of substitution (~ 0.1 mg/ml) was inadequate to obtain significant increases in specific activity of tyrosine hydroxylase. Methods were developed which showed that heparin fixed to the Sepharose 4B bead was available as a polyanion and could be measured by interaction with methylene blue under our conditions. Furthermore, a 3 to 4 fold increase in the amount of heparin substituted on activated Sepharose 4B was achieved by subsequent modification of the reaction conditions (1.8-2.3 mg heparin/ml of gel).

The significance of molecular conformations of organized multianions (such as the glycosaminoglycans) in brain function and mental health was not apparent to most ten years ago. However, it was clear then that the structure of inter- and intra-cellular matrices through which neurotransmitters pass, must play a role. The early results demonstrated that small cations were held in specific and highly ordered fashion by various polyanions, described a third parameter of the metachromatic reaction, and set down general techniques for measuring the above kinds of interaction. The findings with cationic polypeptides that such complexes can regulate polypeptide conformation, depending upon the nature of the amino acids neighboring cationic residues, may have direct bearing on the biological aspects of amine storage in the CNS.

Another visible role for basic studies of molecular conformations is seen, for example, in the activation of certain enzymes by heparin. Tyrosine hydroxylase, the enzyme responsible for DOPA formation, is known to occur in both soluble and insoluble forms, of which the former is markedly stimulated by heparin. Furthermore, the enzyme is believed to exist in two forms of "low" and "high" enzymatic capacity. The significance of our studies on the conformational aspects of heparin-polypeptide interactions lies in the quantitative measures of the stoichiometry, which permit the proposal of a molecular model to explain, for example, the activation of tyrosine hydroxylase, and a possible mode of regulation of this enzyme, natural as well as pharmacological. Our studies define a rational approach to selecting agents which might affect this enzyme system in a predicted manner.

During the past twelve months I have studied the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS, reexploring the molecular characteristics and specificity of the interaction using a model for the molecular basis of the activation analogous with that of the antithrombin system. This is characterized in three important ways; 1) specificity of heparin; 2) interaction of heparin with lysyl groups on the protein and 3) a high potency fraction of about 10 percent of the bulk. Partially purified mouse striatal tyrosine hydroxylase, (up to 10 fold purified in a one-step heparin affinity column) was used as the source of the enzyme; heparin fractions, heparan sulfate and other polyanions were looked at in regard to their conformational alterations of polypeptide models. Three new findings of importance emerged, 1) one of our heparin fractions, separated by mass and 5% of the total, is significantly more potent in activating tyrosine hydroxylase on an equivalent charge basis than the bulk preparation; low molecular weight (<6000) and high molecular weight (<28,000) fractions are not active at the same equivalent charge. I have also shown that this fraction has different CD properties from the bulk heparin; 2) heparin sulfate does not activate tyrosine hydroxylase despite its heparin-like components, nor does sulfated keratan sulfate, another glycosaminoglycan of the heparin class (1969); and 3) a potent inhibitor of the enzyme was developed; this provides a one-step affinity column purification of 5-10 fold when coupled to Sepharose B, similar to that of the heparin-substituted affinity gel. This enzyme preparation (A-TH) has a Ki for the inhibitor 8-fold lower than that of the heparin affinity preparation (H-TH).

The analogy with the antithrombin system is partially supported by the results. The exception is that heparin appears better to relieve inhibition (desensitize the inhibited enzyme to the fully activated state) than to cause the direct activation.

The H-TH preparation of tyrosine hydroxylase has been repeatedly shown not to exhibit substrate (tyrosine) inhibition with tetrahydrobiopterin cofactor. Furthermore, the inhibitor markedly raises the Km for tyrosine from about 15 to 125 μMolar. Kinetic studies indicate that it is a mixed type of competitive inhibition. Heparin in desensitizing the enzyme from the inhibition, causes a decrease in the apparent Km for tyrosine towards that of the activated form. It may be significant that the fasting blood level of tyrosine in the C57Bl/6N mouse is about 80 μMolar, in between the above Km values. These results bear on possible regulatory properties of tyrosine hydroxylase *in vivo*, especially in regard to transitions between forms of low and high enzymic capacity.

In collaboration with a Visiting Scientist, Dr. P. Righetti, we studied the mechanism of the anomalous isoelectric focusing of heparin using our metachromatic methods for measuring heparin-amine interactions. We showed that heparin formed complexes with the carrier ampholytes which varied in stoichiometry with pH, heparin concentration, and heparin-to-ampholyte ratio, regardless of molecular weight (except in the two extremes of the range). Thus, heparin molecules were immobilized by binding to carrier ampholytes in the various pH zones, and a general mechanism of banding of various polyanions upon isoelectric focusing was elucidated. This system is being explored as a means of isolating the active heparin fraction.

The proposed course of the project is a continuation of the basic studies of glycosaminoglycan-polypeptide interactions and studies on the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS. Collaboration with Dr. Robert Rosenberg on the optical properties of his "highly-active heparin (antithrombin-binding heparin)" is anticipated. Further quantitation of the tyrosine hydroxylase active fraction will be attempted, and if obtained in adequate quantity affinity chromatography procedures can be modified to yield higher purification with a single column step. The techniques of conformational potential in polypeptides using heparin interaction have been applied to myelin basic protein in collaboration with Dr. Marian Kies, and this will be continued, if space and personnel allow. Because the Institute has found it impossible to set a high enough priority for the allocation of funds for the purchase of a spectropolarimeter for use in this project, further experiments involving circular dichroism must continue to be conducted in another laboratory at NIH, at not inconsiderable extra cost in time and effort.

Honors:

Nomination for membership to NIH Scientific Review Groups. Invited speaker, Hypertension-Endocrine Branch, NHLBI (Biochemical Pharmacology Section), June, 1979. Invited participant and speaker at the 1980 meeting on The Chemistry and Biology of Heparin on March 21-22 organized by Dr. W. Vivgil Brown, Mount Sinai Medical Center, N.Y. and Dr. Kenneth G. Mann, Mayo Clinic, Rochester, N.Y.

Publications:

Righetti, Pier Giorgio, Brown, Ronald P. and Stone, Audrey L.: Aggregation of carrier ampholytes on heparin and other acidic polysaccharides in isoelectric focusing. Biochim. Biophys. Acta 542: 232-244, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01037-11 LNC

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Role of the Cell Membrane in Cellular Organization, A Molecular Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Neville, Jr.	Chief, Sect. on Biophys. Chem.	LNC NIMH
OTHER:	Wyrta Heagy	Staff Fellow	LNC NIMH
	Richard Youle	Staff Fellow	LNC NIMH
	Gary Murray	Visiting Fellow	LNC NIMH

COOPERATING UNITS (if any)

National Institute of Arthritis, Metabolism and Digestive Diseases

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of a toxin and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents.

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved in the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Clinical Applications:

A hypothetical example of the clinical utility of an artificial hybrid protein reagent is the following. Many malignant tumors are known to carry tumor specific receptors on their cell surfaces (tumor specific antigens). An artificial hybrid protein containing a functional group binding to these specific receptors and linked to a highly toxic intracellular functional group (diphtheria toxin fragment A) might function as a tumor specific toxic reagent. The advantage of this type of reagent over currently available antitumor drugs would be the property of cell type specificity.

Artificial hybrid protein technology also holds the promise for constructing new types of neuropharmacologic reagents. Tetanus and botulinum toxins inhibit the release of inhibitory and excitatory neurotransmitters respectively, by virtue of their intracellular functional A chains. By linking these A chains to proteins which bind to only certain areas of the brain, a high degree of regional specificity might be achieved, and it is likely that the regional specificity would be mirrored by behavioral specificity.

Major Findings:

1. The major premise underlying hybrid protein technology is that a surface receptor which mediates the entry of a specific protein can also mediate the entry of other proteins linked to the specific protein, and

that after entry the linked protein remains functional. This premise has been verified in our laboratory by Dr. Richard Youle who has shown that the hybrid ricin-diphtheria toxin fragment A can enter via the ricin receptor with resulting ADP-ribosylation of intracellular peptidyl elongation factor II catalyzed by the diphtheria toxin A fragment.

2. The major aim of hybrid protein technology is to achieve the construction of a cell type specific reagent. This has been accomplished in our laboratory by Dr. Richard Youle and Dr. Gary Murray.

It is known that fibroblast lysosomal hydrolases gain entry to fibroblasts through receptors which bind mannose-6-phosphate residues on the hydrolase. Drs. Murray and Youle have linked monophosphopentamannose residues to ricin and assayed the toxicity of this material (M6P-ricin) in the presence of lactose which blocks the binding of ricin to its own cell surface receptor. In the presence of lactose, M6P-ricin enters fibroblasts via the mannose-6-phosphate receptor. Entry by this route does not occur with HeLa cells or human amnion cells which apparently lack the receptor entry system. Consequently, under these conditions, fibroblasts are 8 and 13 times more sensitive to the toxic effects of the ricin A chain than amnion and HeLa cells.

These findings show that it is possible to construct cell type specific reagents by utilizing artificial hybrid protein technology. The overall goals of the project therefore appear to be sound.

Publications:

Youle, R.J. and Neville, D.M., Jr.: Receptor-mediated transport of the hybrid protein ricin-diphtheria toxin fragment A with subsequent ADP-ribosylation of intracellular elongation factor II. J. Biol. Chem. in press.

Youle, R.J., Murray, G.J. and Neville, D.M., Jr.: Ricin linked to phosphomannan binds to fibroblast lysosomal hydrolase receptors resulting in a cell type specific toxin. Proc. Natl. Acad. Sci. USA in press.

Kahn, R.C., Goldfine, I.D., Neville, D.M., Jr. and DeMeyts, P.: Alterations in insulin binding induced by changes in vivo in the levels of glucocorticoids and growth hormone. Endocrinology 103: 1054-1066, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01038-08 LNC

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of Mutant Mice for Phenylketonuria and Dihydropteridine Reductase Insufficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone Research Chemist LNC NIMH

COOPERATING UNITS (if any)

Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	none

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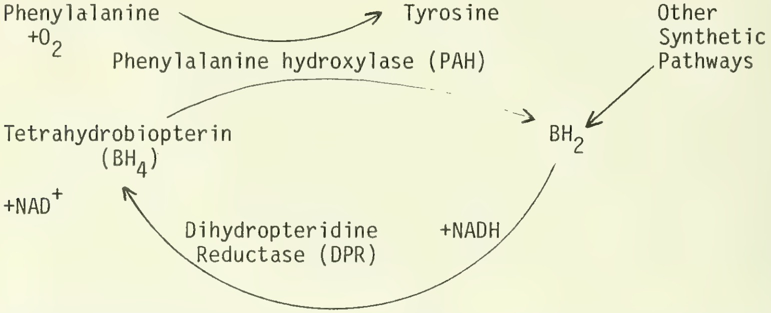
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is a small program to develop a murine model for PKU and/or related diseases. Another objective is the elucidation of regulatory mechanisms in the expression of phenylalanine hydroxylation (PH). Criteria for a putative deficiency in PH were established: plasma phenylalanine levels, plasma phenylalanine-to-tyrosine (P/T) ratio, and hepatic levels of dihydropteridine reductase (DPR). Early results indicating that hepatic DPR in mice was not in great excess were contrary to generally accepted hypotheses. DPR insufficiency was expressed by mild symptoms of deficiency in PH. These findings pointed out inadequacies of standard programs for detecting heterozygote mouse PKU mutants. Murine DPR insufficiency was studied using mildly elevated P/T as a criterion. Putative mutant families were developed, but infertility and/or selective survival led to a loss of viable abnormal offspring at F_4 or higher. Hepatic enzyme and cofactor patterns differed among the families, a reflection of the complex nature of PH. Current work elucidates the PH system in mice, shows the relationship of in vivo elevated P/T to in vitro measurable hepatic phenylalanine hydroxylase (PAH) using parachlorophenylalanine to inhibit PAH in vivo, and explores pharmacologically-induced murine models for dihydropteridine reductase deficiency.

Project Description:

The lack of appropriate animal models for the study of enzyme-deficient genetic diseases associated with mental retardation has checked the search for the pathophysiology directly relating the CNS lesion with the enzyme defect. This project was started as a single-laboratory size program to obtain a murine model for the human disorders related to phenylketonuria (PKU). Suspect heterozygote mutant offspring were generated using double-dose X-irradiation of mouse sires (see previous reports, M-NC-8, 1972-74). The offspring were screened for the defect in a manner similar to the human screening program, using blood plasma levels of phenylalanine (P), tyrosine (T), and their ratio (P/T) to distinguish abnormal, high values in possible mutants from a distribution of values obtained from mice with normal phenylalanine hydroxylation (PH) systems here shown:



The scheme illustrates the complex nature of the enzyme systems governing the steady-state levels of the amino acids in the blood (two enzymes, a cofactor and a coenzyme). Thus, the screening program, in principle, would detect a deficiency in any component shown or a change in amino acid levels due to other causes. At the onset of the project the dogma indicated that dihydropteridine reductase (DPR) was in such excess as to permit normal levels of hydroxylation in the heterozygote mutant deficient in this component. The program included, therefore, screening of a large sample of liver biopsies for direct measurements of DPR in the liver. Methods for screening for total dihydrobiopterin in hepatic samples were also used in this project.

Over the course of this and the previous year the project has not received sufficient support or personnel to carry out its aims vigorously. The current thrusts of the project are 1) to achieve a pharmacological model for the suspect PKU mouse in order to obtain the proper criteria for screening; and 2) to complete in vitro kinetic studies of the new DPR inhibitor (see Z01 MH 01038-07 MH, 1977-78) and to utilize this drug in studies of pharmacologically-induced DPR deficiency. The difference in susceptibility to m-fluorophenylalanine (mFPA) (shown in rats) of normal and PH deficient animals as a possible method for selection of phenylalanine hydroxylation-deficient mice by using a drug whose metabolism utilizes the PAH system to form a highly toxic mitochondrial poison, fluoroacetate, is now assessed as too "uncertain" and is not recommended. It had been shown by others that rats treated with parachlorophenylalanine (pCPA) were protected from the lethal effects of high doses of the convulsant drug for 8-11 hours. pCPA is long-known to induce a state of about 95% PH deficiency in the rat for 24-72 hours after a single injection of about 360 mg/kg. Attempts to induce a similar state in mice had failed to produce more than 50% inhibition of PAH for short periods, at the same or double that dose. This project applied various modes of administration of pCPA to mice, and measured the time course of uptake of the agent into blood plasma and liver, with corresponding in vitro enzyme assays for hepatic PAH. A program for separation of phenylalanine, tyrosine and pCPA from blood plasma and liver extracts at various times after administration of reagent was developed for the automated amino acid analyzer in collaboration with Gladys Deibler, LCM, NIMH. Protein contents of liver samples were determined by colorimetric analysis. Dose response curves for mFPA in the C57BL/6N mouse were obtained by intraperitoneal injection of mice. Comparison of lethality at several levels of PH was made at a mFPA lethality level of about 80%. Various other pretreatments which were expected to interfere with PH were used in attempts to achieve a less than 50% in vitro measurable hepatic PAH level.

Results showed that the difference between the rat and mouse in response to pCPA was due to a difference in uptake and disposal of the agent. Eighteen hours after the administration of 360 mg/kg pCPA in mice scant amount is detected in the blood or liver. The maximum level is reached at 3-6 hours and falls. Doubling the dose leads to approximately twice the maximal concentration in liver and blood from 3-6 hours and falling close to zero by 18 hours. The animals excrete large quantities of phenyl ketones during the 12 hour period. In vitro measurable PAH levels fall during the 6-18 hour period, using the natural cofactor (BH_4) or the synthetic ($DMPH_4$), confirming our previous findings at 24 hours with $DMPH_4$ (see previous report N-MC-8 1974). The inhibition curve was bimodal between zero and 24 hours. The maximal decrease in hepatic PAH activity compared with normal during 0-36 hours was found to be 45-50%. Plasma tyrosine level decreased, phenylalanine increased and the P/T ratio increased 1.5-3 fold during the zero to 9 hour period.

At the 24 hour level of inhibition of PAH and PH, the dose-response for lethality due to mFPA was not shifted; the 50% deficient animals were, therefore, not less susceptible to the agent. A new mode of administration of pCPA was tried with the view towards achieving high levels of pCPA in the blood and liver over longer periods. This was successful. Maximal plasma levels were about 4 fold higher than previously (approaching the levels seen in rats, 1 mM) and persisted over 24 hours. Tests for the protective effect of pCPA-induced PAH deficiency in mFPA toxicity in mice were undertaken, and reported last year (see Z01 MH 01038-07 LNC, 1977-78 for details).

Continuation of the study of the remaining, aged nonfertile 4th generation of suspect DPR-mutant families exhibited diminished in vitro measurable PAH activity (25-80% of controls)(see previous reports MNC 8, 1972-76). It was further shown that in vitro measurable PAH activity was similar in young adults and aged exbreeders from the normal NIH colony. The data further confirm the original premise that small increases in P/T in mice is a reflection of diminished PH activity in vivo due to either DPR insufficiency or decrease in the in vitro measurable PAH. The suspect mutants, therefore, have been established to exhibit enzymatic and biochemical characteristics of the human genetic disorder of Dihydropteridine Reductase Deficiency. We explain the failure to maintain the substrain by the normal reproductive methods as due to impairment of the hormonal regulations of mating, nesting, fertility and/or lactation/care behavior in mice since both male and female affected animals often survived with the deficiency beyond one year of age. In one case of 8, hormone treatment induced fertile mating and viable offspring.

Current results found that the new DPR inhibitor was an order of magnitude more potent in vitro than methotrexate using the natural cofactor BH_4. This indicates its eminent suitability for in vivo administration and inhibition of DPR to produce DPR deficiency in mice, with the caviat that folate intermediates be administered at the same time.

The results are significant to an understanding of the effects of pCPA in mice and modes of inhibiting PH in vivo in mice. The methods and results will be reported in a joint manuscript in progress with Gladys Diebler, LCM, NIMH. Parameters of PH insufficiency in mice such as P/T ratio are now quantitatively related to the degree of impairment of hepatic PAH. Results confirmed the value we have used of 1.6 and over, as suggestive of heterozygosity for "PKU" in suspect mutant mice. P/T values of about 2.0 are very elevated in this species where values greater than 3.0 have never been observed in PH deficient animals.

The studies of murine DPR deficiency (and preparation of appropriate manuscripts) will be continued, - space, equipment and personnel permitting. A pharmacologically-induced state of DPR insufficiency in mice will be studied with concomitant changes in hepatic PAH level and/or CNS effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01081-09 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Role of Motor Cortex in Control of Voluntary Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. V. Evarts	Chief	LNP	NIMH
	Y. Shinoda	Guest Worker	LNP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.08	0.83	2.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves utilization of single neuron recording techniques in behaving monkeys to study brain mechanisms underlying volitional motor performance. Monkeys are trained to make precise movements of a handle whose rotation controls a visual display. There is intense motor cortex activity for even the smallest of these voluntary movements, with the proportion of motor cortex neurons involved in the control process being much greater than the proportion of spinal cord motoneurons involved in such movements. We have found that there is a reflex negative feedback pathway which stabilizes outputs of motor cortex neurons controlling small precise movements, but that there are internally generated central programs which can overcome reflex feedback when a subject wishes to carry out a movement which runs counter to "hard-wired" reflex inputs.

PHS-6040
(Rev. 10-76)

Project Description:

Monkeys are trained to make precise movements of a handle whose rotation controls a visual display, with reward being given to the monkeys according to the precision of their performance. Microelectrodes are introduced into the brain to record impulses of motor cortex cells during these precise movements.

Major Findings:

Results obtained in this experiment reveal a striking intensity of motor cortex activity for even the smallest of the monkey's voluntary movements. Furthermore, the proportion of motor cortex neurons discharging in control of precise fine movements is much greater than the proportion of spinal cord motoneurons involved in such movements. Thus, within a zone of motor cortex controlling hand movements, a large proportion of the cortical neurons participate in controlling a rather small proportion of the hand muscle motoneurons. And the motoneurons controlled are those producing the finest, most precise movements. As larger movements (requiring more intense muscle discharge) occur, additional motor cortex neurons are recruited, with some motor cortex neurons remaining silent with small movements and then becoming active only as muscle contraction becomes more intense.

The results of this project have shown that a second motor cortex feature underlying its role in precise motor control is the existence of a high gain negative feedback servo system which regulates motor cortex output automatically, with this feedback being preferentially focused on neurons controlling the smallest, most precise movements. The anatomical pathways bringing negative feedback to motor cortex are currently being worked out in detail in another project within LNP (Project No. Z01 MH 01092-01).

Central programs which override the servo. The patterns of motor cortex discharge referred to above are those which are of critical importance in generating and stabilizing our finest, most precise movements. But what happens when a subject wishes to carry out a movement which runs counter to "hard-wired" reflex inputs? In order to gain insight into this matter, we have studied motor cortex neuron discharge in monkeys trained to react to limb displacements according to prior cues which sometimes called for muscular contractions opposite to those which would have resulted from servo controls. To clarify the monkey's task in this situation, let us consider the case of a person who is standing upright and who is instructed to lean forward (without moving his feet) when the experimenter subsequently displaces him. In this situation the subject will first get set to move forward and then await the impending displacement. If the displacement is then one which by itself moves the subject forward, the subject's reflex postural response (necessary for maintenance of equilibrium) will be to lean backward rather than forward as instructed. In order to lean forward as instructed, the subject must shift out of the closed-loop mode necessary for maintenance of the stable, upright posture--and shift into an open-loop mode to generate a centrally programmed movement so as to lean forward as instructed (into a net or the waiting arms of someone who will catch him when he falls).

To study an analogous situation in the monkey, monkeys were trained to begin by positioning a handle precisely and holding it immobile for a few seconds. During this period of immobility, motor cortex output was regulated by afferent input in the negative feedback closed-loop mode. Then a red or green lamp was illuminated--telling the monkey how to react to a subsequent displacement of the handle--red meaning that the handle displacement was a signal to pull backward and green meaning that it was a signal to push forward. The monkey was rewarded for reacting to handle displacement according to the red or green cue. In records of motor cortex nerve cell discharge obtained in this paradigm, it was seen that servo control of motor cortex was cut off within 40 msec of the handle displacement signal which was a trigger for the "pre-programmed" pull or push movement that the monkey had gotten set to perform upon receiving the red or green cue. Within this brief 40-msec period, the control of motor cortex was abruptly shifted from the closed-loop system underlying postural stability to the open-loop system underlying the pre-programmed movement.

Subcortical origins of motor programs. While the cortical sensory areas for the hand have a role in relaying signals which control motor cortex output in the closed-loop mode, these same cortical areas do not provide the signals which underlie movements that occur in spite of rather than because of the reflex consequences of sensory input. The inputs underlying motor cortex discharge in pre-programmed movements reach motor cortex from the cerebellum via the thalamus. Direct evidence showing that the cerebello-thalamo-cortical pathway is involved in centrally programmed open-loop control has been provided by the LNP work in which we recorded activity of single nerve cells in dentate and interposed nuclei of the cerebellum of the monkey in the same paradigm referred to above--the paradigm in which pre-programmed arm movements were triggered by displacements of the arm which was to move. It was found that motor set (as established by the red or green cue) had profound effects upon the responses of most cerebellar dentate neurons, with the "set-dependent" activity beginning as early as 30 msec after the signal to move. Such dentate activity, dependent upon prior instruction and occurring at 30 msec, has time to traverse the thalamus and generate centrally programmed activity beginning at 40 msec in the motor cortex.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in basal ganglia and cerebellum.

Of the two major classes of inputs impinging upon the cerebral motor cortex and generating the stream of impulses passing to the spinal cord, that class of inputs which operates automatically and constitutes the afferent limb of the transcortical servo loop seems the simplest to understand. This transcortical servo operates according to Sherringtonian principles of reflex action. The second set of inputs passing to motor cortex via the thalamus depends upon basal ganglia and cerebellum, and is much more complicated. In order to understand voluntary movement we need to comprehend the sorts of information processed by cerebellum and basal ganglia, and to discover the way in which the outputs of cerebellum and basal ganglia interact in the thalamus. It is these problems which stand at the forefront of studies on brain mechanisms of movement.

Proposed Course:

Thus far in the work on motor cortex, major attention has been focused on a class of motor cortex neurons which send their axons down to the spinal cord via the pyramidal tract. These neurons are referred to as pyramidal tract neurons (PTNs) and are of particular importance since they convey the final output from motor cortex to the spinal cord regions from which impulses pass out to the muscles to generate movement. In experiments currently being undertaken, a second important class of motor cortex output elements will be investigated. These are the motor cortex neurons which send their axons from the cortex to the red nucleus. The red nucleus is a collection of cells which forms a link between cerebral cortex and spinal cord. Unlike PTNs, the cortico-red nuclear neurons (CRNNs) pass only as far as the brain stem, and it is the cell bodies lying in the red nucleus whose axons then pass to the spinal cord to generate motor behavior. Within the red nucleus, impulses from the cerebral cortex are integrated with another set of impulses reaching the red nucleus from the cerebellum.

In the work to be undertaken we will first identify zones within the red nucleus receiving projections from the motor cortex arm area in the monkey. Having identified this area and determined the properties of cells, we will permanently implant stimulating electrodes within the arm area of the red nucleus, and use these stimulating electrodes to excite the axon terminals of fibers reaching the red nucleus from the arm area of the cerebral motor cortex. In these experiments, microelectrodes will be used to record impulses of single nerve cells in the arm area of the motor cortex while electrical stimuli are delivered within the red nucleus to identify that class of motor cortex neurons which sends its axons to the red nucleus. Having identified these CRNNs, we will study their discharge properties during movement and compare them to the discharge properties of PTNs which we have already investigated.

Publications:

Fromm, C. and Evarts, E.V.: Motor Cortex Responses to Kinesthetic Inputs during Postural Stability, Precise Fine Movement and Ballistic Movement in the Conscious Monkey. In Gordon, G. (Ed.): Active Touch. Oxford, Pergamon, 1978, pp. 105-117.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01084-08 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Miles	Visiting Scientist	LNP	NIMH
Other:	S. J. Lisberger	Staff Fellow	LNP	NIMH
	L. M. Optican	Guest Worker	LNP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.9	2.0	1.9

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range aim of this project is to determine the neural basis of adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. One project is concerned with localizing the modifiable elements subserving long-term regulation of the gain of the vestibulo-ocular reflex. Single unit recordings in the vestibulo-ocular pathways of awake monkeys have revealed that the underlying modifications must occur beyond the first synapse in the medial vestibular nuclei. The second project is concerned with the mechanisms by which the static (step) component of saccadic eye movements is appropriately matched to the dynamic (pulse) component to insure stable vision following such eye movements. By recording eye movments and shifting the visual scene after each saccade, it has been shown that persistent post-saccadic retinal image slip is sufficient to induce pulse/step mismatch, suggesting an important role for visual inputs in this adaptive process.

PHS-6040
(Rev. 10-76)

Objectives:

Our overall objective is an understanding of two of the adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. The first concerns the adaptive regulation of the gain of the vestibulo-ocular reflex and our major aim is to locate the site of the neural modifications underlying this capability. Specifically, the present experiments were concerned with the medial vestibular nucleus which contains the cells receiving the vestibular primary afferent input. The second project concerns the process responsible for maintaining an appropriate match between the static and dynamic components of saccadic eye movements and our initial aim was to determine the role--if any--of vision in this process.

Methods:

The first project on the vestibulo-ocular reflex involved single unit recordings in the medial vestibular nucleus of monkeys whose vestibulo-ocular reflex gain had been increased by prolonged exposure to x2 telescopic spectacles or decreased by exposure to goggles providing a visual field which was fixed with respect to the head. During recording, monkeys were awake and seated in a special chair which could be oscillated about the vertical axis by a servo-controlled torque motor. Since the animals' heads were secured to the chair, the chair rotation provided controlled vestibular stimulation. Individual cells were grouped into three classes on the basis of their vestibular- and oculomotor-related discharges and their responses recorded on analog magnetic tape for subsequent digitization and quantitative analysis with a PDP-11/34 computer. The vestibular sensitivity and phase of individual neuronal responses was calculated from the average discharge modulations accompanying sinusoidal oscillations of the chair at varying frequencies.

The second project on saccadic eye movements involved accurate recordings of eye movements using the magnetic search coil technique which has a resolution of few minutes of arc. Monkeys were seated with their heads fixed before a translucent screen on which a densely featured image was back-projected. A servo-controlled mirror galvanometer in the projector's light path allowed the scene to be moved horizontally under computer control. Immediately after each saccade, the computer caused the scene to drift horizontally with an exponential time course (40 ms time constant) by an amount equivalent to 45% of the amplitude of the horizontal component of the saccade. In some experiments the scene was made to drift in the same direction as the saccade and, in others in the opposite direction, thereby inducing consistent post-saccadic retinal image slip.

Major Findings:

1. The discharge characteristics of 1530 cells recorded in the medial vestibular nuclei were examined and over 700 of the vestibular-related cells were subjected to quantitative analysis. Approximately one-half of the cells were recorded in monkeys whose VOR gain was high and the remainder in monkeys

whose VOR gain was very low. Statistical analyses revealed no significant differences between any of the three classes of cells in the two groups of animals in terms of gain, phase or resting discharge rates. Detailed analyses of the individual cell groups also failed to reveal any significantly altered subgroupings, and we conclude that the vestibulo-ocular reflex gain changes must occur beyond the first central synapse in the vestibular relay.

2. After several hours of optically-imposed post-saccadic retinal slip, monkeys developed post-saccadic ocular drift which was always in the same direction as the imposed visual drift and was evident both in the dark and when the animals viewed a stationary visual scene. The time constant of the ocular drift was similar in both situations and averaged about 70 ms. We conclude that the step components of saccadic eye movements are matched to the pulse components by adaptive mechanisms which, at least in part, sense post-saccadic retinal image slip.

Significance to Biomedical Research and the Program of the Institute:

A great deal is known about the anatomy and physiology of eye movements. The discrete, rather machine-like quality of the oculomotor system has proved particularly amenable to the analyses of information processing at the single neuron level. The fact that the system also displays plasticity opens up new approaches to the subject of motor learning at the cellular level in the primate central nervous system. The single unit recording studies are being undertaken to establish the exact locus of the modifiable elements in the vestibulo-ocular reflex, a necessary first step before it will be possible to investigate the underlying cellular mechanisms. The studies on the saccadic system are at an earlier stage of development and have established the potential significance of visual inputs in the adaptive regulation of these eye movements. The latter may prove to be of clinical significance since the kind of disruptions which we have induced by purely visual means are seen in some pathological cases (for example, VIth nerve palsies of recent origin) and in some strabismic patients recovering from corrective surgery.

Proposed Course:

The single unit studies will continue to search for the locus of the modifiable elements in the vestibulo-ocular reflex and will concentrate on the later stages of the well-known disynaptic vestibular pathway. The saccadic eye movement studies will examine the time constant and significant input parameters of the newly discovered, visually-mediated adaptive mechanisms matching the pulse and step components. Single unit recordings will also be initiated in the floccular lobe of the cerebellum, lesions of which are known to disrupt this adaptive capability.

Publications:

Miles, F.A., and Evarts, E.V.: Concepts of motor organization. Ann. Rev. Psychol. 30: 327-362, 1979.

Lisberger, S.G., and Fuchs, A.F.: Role of the primate flocculus during rapid behavioral modification of the vestibulo-ocular reflex. I. Purkinje cell activity during visually guided horizontal smooth pursuit eye movements and passive head rotation. J. Neurophysiol. 41: 733-763, 1978.

Lisberger, S.G., and Fuchs, A.F.: Role of the primate flocculus during rapid behavioral modification of the vestibulo-ocular reflex. II. Mossy fiber firing patterns during horizontal head rotation and eye movement. J. Neurophysiol. 41: 764-777, 1978.

Lisberger, S.G. and Fuchs, A.F.: Role of the Primate Flocculus During Rapid Behavioral Modification of the Vestibulo-ocular Reflex: Clinical Implications. In Kommerell, G. (Ed.): Disorders of Ocular Motility. Munich, Verlag, 1978, pp. 147-151.

Fuchs, A.F., Evinger, L.C., King, W.M., Lisberger, S.G. and Baker, R.: Single Unit and Lesion Studies on the Monkey MLF. In Kommerell, G. (Ed.): Disorders of Ocular Motility. Munich, Verlag, 1978, pp. 153-157.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01090-03 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of Central Nervous System Functional Anatomy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Miles Herkenham	Staff Fellow	LNP	NIMH
Other:	Candace Pert	Pharmacologist	BP	NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.0	1.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neuroanatomy research laboratory has been used this year for studies in the a) tracing of neuronal fiber connections in the brain, b) development of new in vitro autoradiography technique for localizing opiate and other peptide receptors in the brain, and c) localization of brain sites which are selectively involved in sleep and drug-induced sedation. The fiber-tracing technique, based on transport of tritiated amino acids, has been used to show by autoradiography a variety of laminar distributions of thalamic projections to cerebral cortex. These data provide structural evidence for mechanisms of thalamocortical integration. In addition, a new method for in vitro localization of brain receptors within slices of fresh-cut brain tissue is being developed. The technique has the potential for providing a simple and general way of visualizing sites of action of a wide range of biologically active substances in any fresh brain tissue. Receptor sites for opiate derivatives and benzodiazapines are currently under investigation. A third application of the autoradiographic technique will be to visualize brain areas with special functional and metabolic characteristics. Labeled 2-deoxy glucose may reveal brain sites that have special metabolic properties during sleep states following various forms of experiences by rats.

I. Studies of CNS Connections

Objectives:

This project uses histochemical tracing techniques to map neuronal connections within the brain. Work in progress has dealt with the cortical projections of thalamic nuclei, which collectively may be called the "non-specific" thalamus. The cell groups under investigation--the midline, intralaminar, parafascicular, ventromedial and ventrolateral nuclei--attracted interest over 30 years ago when electrophysiologists found that electrical stimulation of nonspecific thalamic sites in the cat could elicit widespread and synchronous cortical responses. The data further suggested that the pathways carrying these electrical events to the cortex were not only widely distributed, but were anatomically separate from the known thalamocortical pathways originating in the "specific" thalamic relay nuclei. Moreover, microelectrode depth recordings suggested that specific and nonspecific afferent fibers terminate in separate cortical layers. Unfortunately, however, the neuroanatomical tract tracing techniques, then based on degenerating axons, could find little evidence for nonspecific thalamocoratical projections terminating in wide areas and in unique laminar patterns. Consequently, the early workers abandoned their interest in the possibility that these mechanisms could in part underlie cortical and behavioral arousal phenomena, and even conscious integration. The present studies have provided the long-sought anatomical bases for the electrophysiological phenomena.

Methods Employed:

The techniques of anterograde fiber tracing by tritiated amino acid autoradiography and location of cells of origin by retrograde axoplasmic transport of HRP, both developed in the last decade, have provided more sensitive and artifact-free data than the older degeneration methods. Moreover, the deposition of the tracer enzymes in the brain by microiontophoresis produces injection foci that are much smaller, yet more concentrated, than those produced by hydraulic pressure. The combination of these newly developed methods has made possible the aforementioned research.

Most of the work done this year has been autoradiographic. Essential facilities were installed in August 1977 and have been in full operation since then. In addition, a technician has been trained to perform careful and ultraclean histology necessary for producing good autoradiographic material.

The anterograde data can be corroborated by using the HRP technique to locate cells of origin of widespread thalamocortical projections. Placement of the retrograde marker into various cortical regions will label nonspecific thalamic neurons only if the histological procedure is maximally sensitive. Efforts are in progress to insure that reliable, sensitive results can be obtained. Several staining protocols can be followed to bring out the visible HRP reaction products, and some call for more sensitive and safer (i.e., noncarcinogenic) substrates than others. Since these are the more recently developed methods, certain reaction vicissitudes must be eliminated by experimentation with the procedure. Consideration has been given to the likelihood that the technique will later be used for studies of the monkey brain, which presents additional histological problems because of its larger size.

Major Findings:

Previous work by the principal investigator utilizing the autoradiographic technique has shown a remarkable and unique projection to the most superficial layer of almost the entire neocortex originating from the ventromedial thalamic nucleus in the rat. Another widespread and hitherto unrecognized projection to the hippocampus and limbic cortices was found after placing a mixture of ^{3}H-leucine and ^{3}-proline into the nucleus reuniens of the thalamic midline group. Results from autoradiographic cases of isotope injection into intralaminar thalamic nuclei suggest another instance of widespread neocortical distribution but, in contrast to that of the ventromedial nucleus, intralaminar projections terminate in deep cortical layers. Some results suggest that thalamic inputs may be distributed to two or even three layers, and current experiments are aimed at determining whether the multiple and spaced laminations arise from a single or adjacent thalamic cell groups. Further analyses will also provide the topography of the thalamocortical and thalamostriatal projections. Most of this information can be acquired from HRP case material.

An unexpected finding from the control cases, in which the ventroanterior and ventrolateral nuclei were injected, is that those nuclei previously thought to project to the motor cortex appear to have a much wider cortical distribution. Autoradiographic and HRP experiments are now underway to map the projections of this additional thalamic complex, which is also associated with the nonspecific projection system. Interest in the inputs to motor cortex may generate research in these same systems in the monkey, thereby providing a bridge between the anatomical work in the rat and some ongoing and planned electrophysiological/anatomical studies concerning the primate motor cortex.

II. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations
Objectives:

Since the autumn of 1978 an extensive collaboration has been undertaken with Dr. Candace Pert and her coworkers in the Biological Psychiatry Branch of the NIMH. Our efforts have been aimed at developing new methods to localize specific brain regions, the sites of action of pharmacologically active drugs, and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and the fiber pathways and terminal distributions in any given region. This search for anatomical correlates is augmented by the use of immunocytochemical mapping techniques for selectively marking cells and axons containing the putative endogenous transmitters that are released onto the receptors. The assignment of a neurotransmitter to each of the identified CNS pathways is the ultimate goal of this type of effort.

Methods Employed:

One method for localizing receptors to specific brain regions is to assay fresh pieces of brain tissue dissected out on a regional basis. The radioassay technique was used for a series of experiments in which the neocortex of the rat was stripped into its constituent layers and each layer was analyzed for its content of several peptide receptors. This method is tedious and slow, so a subsequent major effort has been to develop an autoradiographic technique for labeling receptors on brain slices and visualizing their locations microscopically. At present we can successfully attach fresh cryostat-cut brain slices to glass slides, freeze-dry and then incubate them in solutions containing a labeled ligand. Excess and nonspecifically bound ligand is washed off in cold rinses, then the slides are lyophillized again. The sections are fixed and rinsed, then dipped in radioactive-sensitive emulsion for autoradiography. Retention of the label on the section is monitored at each step by liquid scintillation counting.

Major Findings:

The radioassay of dissected cortical layers has revealed the laminar distribution of receptors for bombesin, vasoactive intestinal peptide (VIP), diazepam (Valium) and β-noradrenalin (clonidine). The most exciting result is the high concentration of VIP receptors in layers I and VI of the cortex, since these are areas that receive the nonspecific thalamocortical projections described earlier in this report. Thalamic lesions have been made unilaterally to assess the receptor content after deafferentation of the thalamic inputs, but so far the results have been equivocal: one study showed apparent receptor supersensitivity on the lesion side, but subsequent replication attempts failed to do so.

The *in vitro* autoradiography method is in a final stage of development. The problem of fixation of the ligand to the receptor is the last hurdle being worked on. If the fixation is unsuccessful, the method of autoradiography for diffusable substances will be used. At any rate, we will soon have a method for fast and simple marking of receptors by autoradiography in brain slices taken from fresh, unfixed tissue. Since the discovery of the first brain receptors only a few years ago, numerous investigators have attempted to show their localization in brain. With the *in vitro* method, all of the past studies could be replicated in a few months, and progress in the fields of comparative and developmental receptor anatomy would be rapid.

III. The CNS and Sleep

Objectives and Methods:

The uptake of tracer amounts of ^{14}C-labeled 2-deoxy-D-glucose (2DG) by brain tissue reflects glucose utilization of a particular region (see Project No. Z01 MH 01085-04 LNP for the method). A general lowering of brain metabolism by anesthetics occurs for most brain areas, but in this project it has been found that an anatomical system consisting of the habenula, interpenduncular nucleus and the habenulo-interpenduncular tract, shows relatively much greater labeling by ^{14}C-2DG during anesthesia. This demonstrates altered functional activity in an anatomically defined neuronal network including the interconnecting myelinated fiber tract.

Similar states of altered metabolism may occur during sleep. Current behavioral investigations done in collaboration with Dr. Martin Zatz in the Laboratory of Clinical Science in the NIMH are indicating that carbachol, a drug that mimics the action of the neurotransmitter acetylcholine, can produce profound and longterm changes in the activity and sleep-wakefulness cycle in rodents. The pharmacological and behavioral data can provide clues to the neurochemical mediators for circadian rhythms. Alterations in physiochemical states in the brain may be measured by two techniques already described in this report--one by the 2DG method and another by the in vitro autoradiography method. The applications of these techniques must await the outcome of the behavioral experiments designed to demonstrate the role of acetylcholine in sleep cycles. Alternatively these methods might be used to show some of the CNS functions during sleep as a consequence of prior experience. Such experiments are in the planning stage.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The study of thalamocortical projections, when complete, will show more precisely the nature of thalamic terminations in the cerebral cortex. The newly developed neuroanatomical histochemical tracing methods, wherever applied, tend to reveal CNS connections in more detail and entirety than was previously demonstrated or imagined. In this case, they show that a traditional concept--that thalamocortical fibers terminate mainly in the intermediate layers--must be revised in light of the demonstration that the cell groups lying outside the main thalamic relay nuclei have unique forms of laminar afferentation. Just as the "wiring plan" of the cerebral cortex must include the lamination of specialized cortical efferent cells, now the various types of laminar inputs must be included for a better understanding of cortical function.

Likewise, the great spread of these nonspecific thalamic projections over many distinct cortical regions suggests an important form of neural integration. The emerging possibility that the widespread projections arise from thalamic nuclei which are themselves innervated by components of the extrapyramidal system (substantia nigra, cerebellum, corpus striatum) suggests some role of movement in states of awareness and arousal. The demonstration of similar neural connections in higher mammals would be useful.

The study of the brain localization of radioactively labeled substances such as 2-deoxy-D-glucose, peptides and diazepam, is an emerging hybrid of recently developed histochemical and biochemical techniques. In each instance the knowledge to be gained permits new insights into functional neuroanatomy. Previous efforts to elucidate structure-function relationships were limited by the paucity of useful techniques. Autoradiographic localization of biologically and pharmacologically active substances provides a more powerful tool for observing how the central nervous system operates.

Publications:

Herkenham, M. The connections of the nucleus reuniens thalami: Evidence for a direct thalamo-hippocampal pathway in the rat. *J. Comp. Neurol*. 177: 589-610, 1978.

Herkenham, M.: The afferent and efferent connections of the ventromedial thalamic nucleus in the rat. *J. Comp. Neurol*. 183: 487-518, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01091-02 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Motor Function in Patients with Neuropsychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. V. Evarts	Chief	LNP	NIMH
OTHER:	D. B. Calne	Chief	ETB	NINCDS
	H. Teravainen	Visiting Scientist	LNP ETB	NIMH NINCDS

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.35	1.5	1.85

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relevance of movement disorders to neuropsychiatric illness has become increasingly apparent with the discovery that symptoms of Parkinson's disease may occur as side effects during therapeutic administration of neuroleptic drugs and that cessation of neuroleptics may be followed by tardive dyskinesia. The potential value of information concerning such neurochemically determined motor disorders is twofold: 1) at an applied level, quantitative measures of motor function will be useful in drug dosage regulation of therapeutic agents and in drug effectiveness comparisons for different agents; 2) at a basic level, discovery of mechanisms of motor disorders will help us to understand the brain disturbances underlying the wide range of neuropsychiatric illnesses related to neurochemical disturbances. Studies thus far have focused on obtaining quantitative indices of the fluctuations in effectiveness of anti-parkinsonian drug therapy. Tests of movement speed and reflex responses to limb displacement have provided us with measures which correlated with clinically determined fluctuations in drug efficacy, pointing to the feasibility of setting up routine tests of motor function to be administered in clinical settings.

Project Description:

Previous work carried out in this project and described in last year's annual report led us to realize how valuable it would be to develop a computerized system which could be used in large numbers of subjects and which could provide simple, rapidly obtained measures of motor function which would correlate with disease severity and with the effects of therapeutic agents. In the work done on this project in the current year, we have set up a computerized system which will provide us with quantitative measures of motor function useful in describing three aspects of the clinical disturbances which occur in patients with basal ganglia disorders. These are 1) disturbances of walking, 2) abnormal movements (tremor, dyskinesia, chorea), 3) bradykinesia and akinesia.

The work which has been carried out to develop these computerized systems has relied very heavily on collaborative efforts with the Research Services Branch under Dr. Theodore Colburn. In addition to Dr. Colburn, we have collaborated with Wayne Rasband, William Vaughn, and Newlin Morgan.

Major Findings:

Data acquisition and analysis have been set up utilizing a mini-computer, which records experimental results and immediately provides a write-out which can placed in the patient's chart. Using the computerized system, walking is monitored frequently throughout the day in patients receiving medication. The ups and downs of drug treatment are objectively assessed by fluctuations in the gait of the subject. In addition to the test of walking, there is a test of tremor. This test has involved the development of a triaxial accelerometer system which provides measures of the amount of involuntary tremor and athetoid disturbance. Quantitative descriptions of the results of this test will also be immediately available for insertion into the patient's chart. The third aspect of the test measures akinesia and bradykinesia, and will involve both self-paced limb movements and limb movements occurring in a reaction time paradigm in which the subject must await a visual stimulus and make a rapid limb displacement to a preassigned target upon the appearance of the visual stimulus.

In addition to providing immediate quantitative output for insertion in the patient's chart, the motor function testing will provide data stored on computer discs which will be useful in the longitudinal quantitative assessment of patients with neuropsychiatric disorders.

Significance to Biomedical Research and the Program of the Institute:

The methods for motor function evaluation which we have developed will be applicable to large samples of patients and normal control subjects, and will provide data which can be stored in computerized form. As such, the measures to be obtained will be of potential value for studies such as the study currently being undertaken by the NIMH in New Haven. This study, in collaboration with the NIA and NINCDS, is currently underway to select a group of elderly subjects who exhibit psychological and/or neurological disturbances associated with aging. At the present time this study is in its early stages, with initial subject selection to be carried out on the basis of interviews of thousands of persons. In the next phase of the study, neurological examinations will be carried out on those subjects who on the basis of interview appear to have clinical disturbances. It is at this second stage of the study that the NINCDS will enter the picture.

In a third stage of the project, subjects who on the basis of neurological examination fall into categories such as Alzheimer's disease, presenile psychosis, or early Parkinson's disease, will be studied further. It is at this stage that our systems for motor function evaluation would come into play. The systems would provide objective uniform tests of motor function which would make it possible to compare a number of different clinical categories.

Proposed Course:

It is hoped that we will be able to establish a motor function testing laboratory within the ACRF. Of course, it will be a number of years before this is available, but by the time the space is available we should have a great deal of data on the most effective test systems and the most effective computer storage and retrieval systems for assessing motor function in neuropsychiatric disease.

Well in advance of the availability of the ACRF, there will need to be considerable additional planning and coordination between those intramural scientists in Bethesda who will be participating in the study. This planning is currently in progress.

Publications:

Calne, D.B., Kebabian, J., Silbergeld, E., and Evarts, E.: Advances in the neuropharmacology of parkinsonism. Ann. Int. Med. 90: 219-229, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01092-01 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Corticocortical Connections in the Sensorimotor Cortex of the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven P. Wise	Staff Fellow	LNP	NIMH
Other:	Jun Tanji	Visiting Scientist	LNP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.45	1.75	0.7

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of a broad, neurophysiological and neuroanatomical examination of the corticocortical connections of the sensorimotor cortex and their role in primate motor behaviors which rely upon sensory input. Four questions were selected for initial study. First, the influence of the densest of the corticocortical inputs to the primary motor cortex (MI) is being examined by making reversible cooling lesions of the primary somatic sensory cortex (SI) and posterior parietal cortex (area 5), while recording the response of single neurons in the MI cortex to somatic sensory stimulation. Second, the responsiveness of neurons in the primary motor cortex and the supplementary motor cortex (MII), which both receive dense corticocortical inputs from somatic sensory areas of the cortex, is being directly compared in the alert, behaving monkey. Third, the precise responsiveness of neurons in area 3a, the "transition" cortex between MI (precentral cortex) and SI (postcentral cortex), to somatic sensory stimulation and the extent of its corticocortical projection to MI is being investigated. Fourth, the efferent connections of MII, especially its corticocortical and corticothalamic projections, are being examined in an effort to determine the extent to which these connections differ from those of MI.

PHS-6040
(Rev. 10-76)

Objectives:

The somatic sensory inputs to the primary motor cortex (MI) are thought to play a significant role (perhaps by a negative feedback mechanism) in the regulation of precise movements in the monkey. The source of this input is not known and it is the objective of the present project to obtain some information pertinent to this question. Furthermore, there is a more general question concerning the role of sensory inputs to the entire motor cortex (including cortical motor areas other than the primary motor cortex) of sensory modalities other than the somatic sensory. It has long been known that in addition to the MI motor representation, there exists at least one other motor representation (MII, the supplementary motor cortex); and recent evidence suggests the existence of as many as four additional representations. These motor areas may be influenced by somatic sensory or visual inputs, since it is known that each of these areas receives monosynaptic corticocortical inputs from either the somatic sensory cortex or the parts of the posterior parietal cortex related to somatic sensorimotor or visuomotor activities. The present project was formulated in an attempt to determine the significance of sensory corticocortical inputs to various parts of the motor cortex and their role in the production and regulation of the precisely controlled movements characteristic of primates.

Methods:

The monkeys (Macaca mulatta or M. fascicularis) are either (1) trained to make a dorsiflexion (upward) or plantarflexion (downward) movement of one foot upon receiving a visual cue, or (2) adapted to having the foot attached to a foot pedal which passively dorsiflexes or plantarflexes the monkey's foot. A servo-controlled motor establishes the foot position and delivers somatic sensory stimuli which consist either of abrupt changes of torque applied to the pedal or discrete changes of position achieved at a variety of angular velocities. The extra-cellular unit activity of neurons in the somatic sensorimotor cortex (SI, area 3a, MI and MII) and pedal position are monitored, recorded on magnetic tape and analyzed off-line with a PDP-12 computer. For each unit, its relationship with movements and stimuli of two directions (dorsi- and plantarflexion) is determined using the usual correlational criteria. In those experiments involving synaptic inactivation of the postcentral cortex by cooling, the responsiveness of single MI neurons is monitored before, during and after cooling. Following discussions with Drs. Wise and Evarts, the transdural cooling probe was developed for this laboratory by Dr. Stephen Leighton and fabricated by Mr. James Sullivan, both of the Division of Research Services, Biomedical Engineering and Instrumentation Branch. A copper disc (15 mm diameter), attached to the cover of the fluid-filled recording chamber by an insulated stem, is placed on the dura mater overlying the medial aspect of SI and area 5. After an appropriate single neuron in the MI cortex is isolated, ethanol, cooled to 0°C, is circulated over the copper disc. The ethanol cools the copper disc sufficiently to bring the temperature of the cortex, which lies directly beneath the dura, below the 20°C known to be necessary for synaptic transmission. Rewarming is accomplished simply by arresting the flow of cold fluid over the copper disc and allowing about 15-20 minutes for the vascular system to restore the cortex to near its normal temperature.

Following the recording procedures, small amounts (10-20 uCi) of [^{3}H]-amino acid are injected into either MII or area 3a. By the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in area 3a and MII can be determined by the tissue autoradiography. These autoradiographic fiber tracing techniques will also allow the accurate reconstruction of the recording sites of each penetration.

Preliminary Findings:

About 500 units have been recorded in two monkeys tested for this project (as of May 1979). Five findings have emerged from these studies. (1) The somatic sensory or posterior parietal cortex (areas 2 and 5) and its projection to MI is necessary for the somatic sensory responsiveness of certain neurons in the MI cortex. Thus, this corticocortical projection may play a substantial role in any feedback mechanism whereby motor behavior is modified or regulated by somatic sensory inputs. (2) Many neurons in MI are not significantly influenced by cooling inactivation of area 2 and 5 and therefore probably receive their somatic sensory input via the transition cortex between MI and SI (i.e., area 3a) or some other thalamic or cortical source. (3) Units in area 3a may be identified by their approximately directly proportional response to a graded series of imposed velocities of foot movement. Such a method of identification of area 3a will allow a controlled neuroanatomical investigation of the cortical and thalamic connections of this cortical area. (4) Though the MII and MI cortex receive a monosynaptic input from almost every known somatic sensory representation in the cortex, neurons in MII are much less responsive than those in MI to peripheral somatic sensory stimuli of the type delivered in this study. Since the peripheral stimulus which we have applied is complex and encompasses many somatic sensory submodalities, it is likely that our finding is a general one. This observation raises significant questions concerning the role of the corticocortical connections between the somatic sensory cortex and MII. (5) Certain observations have led to a modification of the currently accepted view of the topographic organization of the motor "maps" in the parts of the MI and MII cortex concerned with the hindlimbs and tail, and have clarified the relationship of these physiologically defined cortical fields and the cortical fields defined anatomically (cytoarchitecturally), a relationship which has been obscured in the recent past.

Significance to Biomedical Research and the Program of the Institute:

Though it is often assumed in the neurological and neurobiological literature that the different cortical fields relay specific information (e.g., sensory information) between each other via their corticocortical connections, there is very little direct evidence which supports this conclusion. In recent tests of this hypothesis in the visual and the somatic sensory system of the cat, the conclusion has been drawn that the corticocortical connections therein examined relay no specific information between cortical areas. Instead, the corticocortical projections which these investigators examined were thought to exert nonspecific influences on the neurons of other cortical areas. Because of the potential significance of the role of corticocortical connections in sensory information processing, cognition, and motor behavior of primates and other mammals, this neuronal system deserves a rigorous examination.

Proposed Course:

The project described in this report has been recently initiated. Continued experimentation, experimental design, and data analysis will be conducted in this laboratory with various collaborators (Dr. Shinoda and Dr. Weinrich) after the departure of Dr. Tanji. An examination of visual as well as somatic sensory inputs will be incorporated into subsequent experiments.

Publications:

Wise, S.P., Murray, E.A., and Coulter, J.D.: Somatotopic organization of corticospinal and corticotrigeminal neurons in the rat. Neuroscience 4: 65-78, 1979.

Jones, E.G., Wise, S.P., and Coulter, J.D.: Differential thalamic relationships of sensory-motor and parietal cortical fields in monkeys. J. Comp. Neurol. 183: 833-882, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01093-01 LNP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Role of Somatic Sensory Inputs in the Cerebral Control of Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a study of the role of sensory inputs in the cerebral control of motor behavior in the primate. It uses the technique of single neuron recording in behaving monkeys to directly compare the activity of neurons in primary motor cortex (MI) during the performance of learned arm movements with the activity of neurons in the parts of the somatic sensory (SI), parietal and frontal cortex which are reciprocally connected with MI. Two questions form the focus of our investigations. First, the possible modulation of peripheral inputs to the sensorimotor cortex during motor activity is being studied by recording SI and MI response to peripheral stimuli delivered during and before active movements, during different levels of steady state motor activity related to differences in the exerted force or length of the contracting muscles and during isometric versus isotonic contractions in which the animal controls either exerted force or arm position. Second, the relation of posterior parietal and frontal cortical areas to visually guided movements is being investigated by determining whether the neuronal activity in these areas reflect information concerning the visual cue to begin movement, as well as limb position or exerted force.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Peripheral inputs to MI during movement may influence motor activity in several ways. It is the object of the present project to examine two of these possibilities.

1. SI inputs may be rapidly relayed to motor cortex and thus form the incoming sensory limb of a transcortical reflex loop. MI responses consistent with this "servo" hypothesis have been observed when a moving or stationary limb encounters a sudden load. We therefore intend to examine the neuronal activity in those SI areas which are known to be densely and reciprocally connected to MI and determine if the salient input to MI might arise in these regions.

2. Peripheral inputs to MI may also affect motor performance via connections with the posterior parietal cortex (areas 5 and 7). A wealth of neurological, physiological and anatomical data suggests that these cortical areas participate in visually guided movements. One possible mechanism by which this may occur is by association of inputs from visual cortex about the position of a visual target with inputs from SI about the current position of the limbs. Outputs from this area could reach MI by known projections to frontal regions which, in turn, are connected to wide areas of motor cortex. Accordingly, we will examine the behavior of neurons in frontal and posterior parietal cortex which are related to aspects of limb mechanics, such as position or exerted force, and relevant visual stimuli such as a visual cue to begin movement.

Methods:

Monkeys (Macaca mulatta) are trained to turn on the center lamp in a fifteen lamp display by grasping a handle which is attached to a servo-controlled torque motor and either 1) exerting force in a pronating or supinating direction against the handle if it is held in position by the torque motor (isometric mode), or 2) making pronating or supinating movements of the handle if it is free to move (isotonic mode). The visual cue requesting a particular direction of exerted force or active movement consists of a switch from the central lamp to one of the peripheral lamps in the display.

In the isotonic mode, alternating pronating and supinating movements between self-terminated end positions of 80° and 100° are made against a steady torque (.1 newton-meters) which is applied by the torque motor in either a pronating or supinating direction. Stimuli superimposed on these movements are step changes in handle position (position pulses, 50 msec, 8 - 10°) delivered at different phases of the task: 100 msec after the visual cue triggering the active movements (but before any active displacement), during active movement, and during the holding period between movements.

In the isometric mode, the handle position and force exerted by the monkey during steady holding are the same as in the isotonic mode except that the monkey controls force rather than position. Thus, the monkey exerts a pronating or supinating torque of .1 newton-meters against the handle which is locked at a position of 100° or 80°. Stimuli consist of 1) position pulses (50 msec, 8 - 10°) during steady holding and 2) passive limb movements of 20° induced by ramp displacemnts of the handle at a velocity similar to the velocity of active movements in the isotonic mode (150°/sec). Following training of the monkey, a large chamber is attached to the skull which allows single unit activity to be recorded in the extensive cortical regions encompassed by areas 6, 4, 3, 1, 2, 5 and 7 (i.e., the greater part of the sensorimotor and parietal regions of the cortex). Unit activity, handle position, exerted force and stimulus codes are recorded and analyzed off-line by a PDP-12 computer. For each unit analysis focuses on the frequency and pattern of discharge at each steady state force level and handle position, and the change in frequency and pattern of discharge produced by position pulses, active or passive movements and active contractions.

After completion of recording procedures, the animal is perfused and the brain grossly and microscopically examined in order to correlate positions of electrode penetrations with areas of cortex.

Preliminary Findings:

Thus far, approximately 900 units have been recorded in one monkey for this project (as of June, 1979). Three findings have emerged:

1. Of the SI neurons found to be sensitive to passive arm displacements 30% showed a decreased response to isotonic position pulses delivered during active movements (as compared to the responses obtained during the holding period) and in some cases responses to pulses delivered prior to onset of active movements were also decreased. Thus, MI may exert control over its own peripheral inputs via cortico-cortical projections to SI.

2. Although the response of most MI and SI units to position pulses was the same in both the isometric and isotonic modes, the sensitivity of the majority of neurons was affected by both the direction of steady torque and position of the handle. This was the case for several SI units that responded to passive manipulation of the wrist or elbow joints. This suggests that the responsiveness of some SI areas, including those traditionally thought of as receiving primarily joint receptor inputs, may be influenced by cortico-cortical inputs from other SI regions, especially those related to muscle receptors.

3. Several units recorded in what is most likely the frontal cortex (area 6) or posterior parietal cortex (area 7) displayed a striking similarity in their sensitivity to the visual cue to begin movement and to parameters of movement such as direction of exerted force or steady state position. This indicates that these cortical regions may function as true "associative cortex" by relating visual, somatic sensory and possibly other sensory modality inputs to produce a signal which participates in the driving of motor cortex during visually guided movement.

Significance:

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in motor control, more information is needed concerning the characteristics of sensory input to MI, the pathway taken by this input and its role in the initiation and control of motor behavior. As an important step towards this goal, the present project examines several cortical areas through which peripheral inputs could influence motor cortex output.

Proposed Course of Project:

The project described in this report has been recently initiated. More than two years of experimentation and data analysis will be necessary to substantiate the preliminary findings and to provide further insights into the role of sensory inputs in the cerebral control of movement.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01335-09 SMRC

PERIOD COVERED
October 1, 1978 to September 30, 1979.

TITLE OF PROJECT (80 characters or less)

Studies of Schizophrenia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project summarizes the extensive studies with chronic schizophrenics including the development of a rating scale for measuring change over time the therapeutic testing of 5-hydroxytryptophan, tryptophan, alpha-methyl-para-tyrosine, pineal extract, physostigmine, lithium, baclofen, thyrotropin releasing hormone, naloxene, carbidopa, propanolol, as well as many biochemical determinations including monoamine oxidase.

Project Description:

Objectives:

To test existing and create new hypotheses relating to the possible organic basis of schizophrenia. This project attempts to synthesize psychological, physiological, and biochemical disciplines around the subject of schizophrenia.

Methods Employed:

All studies have been carried out in phenothiazine refractory chronic schizophrenics. The drugs were given and measurements made in a double-blind fashion.

Major Findings:

I. The Rating Scales

Nurses' Rating: Experience gained with the rating scale we had been using on the research wards to record clinical status indicated that it had many short-comings. We, therefore, undertook to design a more useful nursing scale that could yield quantitative estimates of the level of psychopathology of chronic schizophrenics. To be useful, the scale had to be objective as possible and be comprehensible to nursing staff trained to the level of nursing assistant.

Numerous conferences were held with the nursing staff to determine which psychiatric concepts were comprehended and could be inquired about on a rating form. Sample forms were devised and tested in the following manner: Every staff member on each shift would complete the rating on two patients. The results of this exercise were then subjected to statistical analysis in order to determine reliability and to find if, in fact, any meaningful consensus had been found as to the meaning of the questions. Different pairs of patients were used and the same patients were tested again to determine if reliability was consistent over time.

We have evolved a rating scale that appears usable by nursing staff trained to the nursing assistant level which will accurately reflect the daily clinical status of the patients. The new nurses' rating scale was tested extensively and various ambiguities eliminated. In an effort to shorten the scale, correlations were run between specific questions. These correlations did not hold consistently in each patient. Thus, no questions could be demonstrated to be expendable. Computor programs for processing the scale have been devised and a new optical scanning machine activated so that quick feedback can be gained from each patient.

The Inpatient Behavioral Rating Scale (IBRS) is an inventory designed to record daily nursing observations of mood and behavior of patients in a clinical setting. It consists of 26 items characterizing a broad range of psychopathology and is particularly suitable for use where change in mood and behavior is relevant to clinical investigation. It is formated on an optical scan sheet for ease of computer entry and analysis. In over a year of use on two clinical research wards, the IBRS has been found discriminating, reliable, and easy to use by the nursing staff.

Rating Scales: We have gained a year's experience using the modified scaled version of the Brief Psychiatric Rating Scale. This scale, originally introduced by Overall and Gorham in 1962, has been extended and descriptive anchor points provided through a joint effort by Dr. Bigelow and Dr. Dennis Murphy. The scale as actually used by nursing staff has been shown capable of reflecting accurately the cycles in a patient with a periodic psychotic illness. Additionally the scale instructions have been shown to be remarkably reliable. This was established by having three psychiatrists of diverse backgrounds, naive to the scale, rate independently 15 patients in an interview setting. The intraclass correlation coefficients obtained from this exercise were quite impressive. We are in process of writing up this scale for publication. Since the original overall and Gorham BPRS has found extremely wide application, we anticipate that our scaled version will gain considerable popularity and application.

Pre-Morbid Rating Scale: During the past six to eight months we have put together a rating scale aimed at assessing pre-morbid adjustment in chronic schizophrenic patients. Items in the scale were either original or adopted and modified items from the Phillips, Gittelman-Klein and Elgin scales. A group of approximately 15 normals (staff of LCP) provided self ratings. To date, approximately 20 chronic schizophrenics from the William A. White Building have been rated from chart material. A total of 44 chronic schizophrenics from Saint Elizabeths Hospital will be rated, along with a larger group of normals. Dr. Phillip Berger is presently using the scale at a VA hospital to provide information on variations in hospital populations and raters. When the scale is eventually able to differentiate subgroups of patients, it will be used in conjunction with the ongoing biochemical studies of chronic schizophrenia.

Premorbid-Adjustment: During the last year, the premorbid scale has been administered to 50 chronic schizophrenics. Following minor wording adjustments to 3 items in order to make the scale more appropriate for normal subjects, 80 normals from the lab personnel, Bolling Air Force Base, and Red Cross volunteers were rated. Blood and, in some instances, urine samples from these subjects were analyzed for substances such as MAO, PEA, and PAA. Upon completion of data collection the relationship of premorbid adjustment to blood and/or urine concentrations of the above compounds will be compared in normal vs chronic schizophrenic subjects. The premorbid scale is also being used to evaluate schizophrenic patients who have been found to have abnormal CAT scans.

Wechsler Adult Intelligence Scale: A previous study has shown that parkinsonian patients treated with L-DOPA had decreased scores over time on the Comprehension subtest of the Wechsler Adult Intelligence Scale (WAIS), whereas scores on all other 10 subtests of the WAIS increased. It was hypothesized that if L-DOPA treatment, which increases dopamine activity in the brain, is directly related to an apparent deleterious effect on the WAIS Comprehension, then a drug such as alpha-methyl-para-tyrosine (AMPT), which decreases dopamine activity, might have an augmenting effect on this subtest. A therapeutic trial of AMPT in a group of chronic schizophrenic patients provided an opportunity to test this hypothesis. It was found that Comprehension scores improved significantly with AMPT. Other clinical rating instruments failed to show any changes.

Scores from 94 psychiatric and neurological patients on the 11 subtests of the WAIS and the Halstead-Reitan Category and Finger Tapping tests were used in a factor analysis. The Category Test had a low loading on the first verbal factor and a highly loading on the second, visuomotor factor; the Tapping Test had a high loading on a small fourth factor of manipulative speed. The finding with the Category Test suggests that it, contrary to the expectation of many of its users, does not distinguish an ability that is both separate from nonverbal intelligence and particularly sensitive to all types of brain pathology. It is concluded that visuomotor and verbal factor scores might be more efficient than conventional scores in studies of effects of brain damage.

II. Serotonin and Its Precursors

A. Behavioral: The study has now been underway for about five years. The first three patients were given alpha-methyl-5-hydroxytryptophan for one month, preceded and followed by one month placebo periods. The active drug made these patients worse.

The subsequent 18 patients were given L-5-hydroxytryptophan together with a peripheral decarboxylase inhibitor (MK-486). MK-486 blocks the conversion of 5HTP to serotonin outside the brain and because MK-486 does not enter the brain makes more 5HTP available for conversion to serotonin within the brain parenchyma. In addition, it is thought that MK-486 reduces 5HTP's side effects.

The patients were given placebo for one month, the active drugs for up to three months, and then a second placebo for one month. Behavioral ratings were done by nurses four times daily. The last 7 days of the highest active drug period were compared to the last 7 days of both placebo periods. For a change to have taken place, we required that the active drug period had to be higher or lower than both placebo periods ($p<0.05$, 2 tailed "t" test). By the criteria indicated, 3 patients have improved, 3 have become worse, and for 12, there was no change. It must be emphasized that these comparisons were made against placebo only and as yet, we have not determined how 5HTP compares to conventional drugs. What is clear is that these drug combinations chemically differentiate at least two kinds of schizophrenic patients; those who become better and those who become worse. Further efforts to determine if these chemical differentiations correlate with any behavioral distinctions are being made.

In order to determine if there are any additive effects of 5HTP and neuroleptics, we are now treating our patients with a constant dose of a neuroleptic, MK-486 and 5HTP (3-6 mg/day). The first three patients were given chlorpromazine as the neuroleptic and appeared to do well. The neuroleptic was then changed to haloperidol. Five male and two female chronic schizophrenics received a combination of haloperidol, L-5HTP and MK-486 in a double-blind, crossover study.

Patients were stabilized for at least one month on an optimal dose of haloperidol and an antiparkinson agent when necessary. After a month on haloperidol and placebo - 5HTP, patients began L-5HTP, 100 mg/24 hours, along with MK-486, 200 mg/24 hours. The intent was to follow a program of stepwise increments of L-5HTP dosage to achieve levels of 3 or more grams/24 hours as in the previous study, but this turned out to be impossible. Of the 7 patients, 3 became so activated on 100 mg/24 hrs of L-5HTP that they had to be removed from the protocol. All 7 patients showed some activation of illness in terms of their underlying pathology or character traits, but in 4 of the 7, these particular reactions subsided quickly, and permitted increased dosage of L-5HTP.

Preliminary data suggest that with each increment of L-5HTP, there was an increment of agitation or an exaggeration of existing pathology. There then followed in some patients an improved clinical status with better integration of thinking and more social interaction. This kind of improvement was vividly reported by 3 patients' families, who were, of course, unaware of the timing of the medication change.

These measurable clinical changes are striking, considering that such low doses of L-5HTP were used. We found earlier that without haloperidol, activation of symptoms occurred only when doses of L-5HTP of 2 to 3 grams per 24 hrs were reached. Tolerance to some side effects of L-5HTP developed in the patients during both studies. Transient parkinsonian side effects occurred in this study, whereas they did not at the much higher levels of 5HTP given in absence of haloperidol. It is clear that haloperidol in some way sensitized the central nervous system to exogenous L-5HTP.

Nine new chronic schizophrenic patients, three females and six males, participated in a second study of the clinical effectiveness of the drug combination 5HTP, chlorpromazine and MK-486. Doses up to 4 gm daily of 5HTP were given along with variable amounts of chlorpromazine. A common daily dose of chlorpromazine was 800 mg; however, patients received 200 mg MK-486/24 hrs. Although daily ratings of patients' attitudes and behavior are not yet available for analysis, no sustained clinical improvement was obvious in any of the patients. A preliminary impression is that 5HTP combined with chlorpromazine, like 5HTP and haloperidol, does elicit an increase in expression of affect. One patient in particular said repeatedly during active drug periods that she felt better, but no lasting change in paranoid ideas was seen. Apparently large doses of 5HTP in combination does not add much to therapy and may produce undesireable displays of irritability or uncontrolled affect in some chronic schizophrenic patients. An occasional patient may experience pleasurable affect on 5HTP without other marked change in clinical state.

Serotonin: L-5-hydroxytryptophan (L-5HTP) and the peripheral decarboxylase inhibitor carbidopa were administered to chronic schizophrenic patients in three separate experiments using a double-blind placebo-controlled crossover design. The three experiments were: (1) L-5HTP administration to 15 patients who had been withdrawn from all neuroleptic medication; (2) L-5HTP administration to seven patients maintained on haloperidol; (3) L-5HTP administration to nine patients maintained on chlorpromazine. Although the groups were diagnostically homogeneous, individual responses were highly variable. Considering each group as a whole, the only significant changes in rated psychosis consisted of an increase in the first group consequent to coming off neuroleptic medication and an increase in psychosis scores associated with adding L-5HTP to chlorpromazine. Neuroleptics apparently sensitize the central nervous system to the effects of L-5HTP loading. Acute exacerbations of psychosis induced by L-5HTP can be reversed by neuroleptics. Our experience does not give encouragement to the hypothesis that schizophrenic illnesses arise consequent to a functional deficit of serotonergic function that can be treated by giving a serotonin precursor in pharmacological quantities.

Lilly 51641: (N-(2-chlorophenoxyethyl)-cyclopropylamine) a selective inhibitor of Type A monoamine oxidase (MAO) activity, was given for 9 to 73 days to eight inpatients with different primary psychiatric diagnoses. After two weeks treatment at daily doses exceeding 200 mg by mouth, this patient group exhibited greater than 90% mean reduction in plasma amine oxidase activity, which persisted for three weeks following discontinuation of the drug. Consistent reductions in platelet MAO were also noted, although the magnitude was less than 25% and such reductions persisted for less than one week following discontinuation of active drug. All 7 patients receiving active drug for longer than two weeks or at daily doses exceeding 200 mg by mouth exhibited significant and substantial mean increases in ratings of agitation or mania. The three female patients seemed especially vulnerable to such excitation during the seek prior to their menses. The only two patients with primary affective illness--one patient with unipolar and one with bipolar depression--had the lowest baseline platelet MAO activities, and showed dramatic antidepressant responses on the drug. In contrast, two schizo-affective patients showed either decreased agitation or increased depression during treatment with daily doses of 50 to 150 mg orally.

The one schizophrenic and one schizo-affective patient with highest baseline platelet MAO were the only two patients who exhibited an increase in psychosis on active drug.

Gross fragmentation of nocturnal sleep and daytime drowsiness was seen in all patients. The circadian rhythm in body temperature decreased in amplitude on the drug. All patients developed postural hypotension on this drug--a side effect which was managed adequately by dosage reduction and temporary bedrest.

B. Sleep Studies: Previously we found that chronic schizophrenic patients had a decreased number of rapid eye movements during REM sleep (REM density) and an increased number in NREM sleep. This situation is similar to one seen after the administration of parachlorophenylalanine (PCPA). PCPA decreases serotonin by inhibiting tryptophan hydroxylase. 5HTP reverses the sleep effects of PCPA-treated patients.

The sleep of 18 male, chronically hospitalized, schizophrenic patients was studied during prolonged 5HTP administration. This was compared to sleep studies of various lengths during the month-long placebo periods and immediately preceding and following 5HTP ingestion. During active drug administration, the peripheral decarboxylase inhibitor, MK-486, was also given. Seven of the 18 patients were studied at intermediate doses, 11 at high doses, and 7 during withdrawal from 5HTP.

EFFECT OF 5HTP ADMINISTRATION AND WITHDRAWAL ON MEAN SLEEP PARAMETERS

	P_1	5HTP (3600-5500mg)	P_1	5HTP (5600-6000mg)	P_1	Withdrawal
No. Subjects		7		11		7
Total Sleep	318	274	335	273***	297	213
Stage II	223	209	219	18*	190	195**
Delta	6	0.04**	19	6***	28	11**
REM Latency	71	192	70	175***	91	19*
REM Time	78	52	86	69***	71	77
REM Density (Index)	1.4	1.6	1.5	1.6	1.5	1.7
REM %	23.2	19.4	24.3	25.2	21.9	36.2*

P1- Sleep during the month placebo immediately preceding drug
Withdrawal - Sleep during the first week following last drug administration
* P .05 (Wilson matched-paired signed rank test).
** P .02
***P .01
Values are in minutes/night except for REM % and REM Density

At low dosages of 5HTP (<2000mg) there was a significant increase in REM Latency (190 vs. 65), but no other changes in sleep. Total REM Sleep and Delta were significantly reduced on high dosages. Patients were agitated and sleep was poor during the first week of withdrawal; however, there was a significant increase in REM % and a decrease in REM Latency. During the second week of 5HTP withdrawal, all sleep measurements returned to baseline.

In addition, at the high dosages, the number of REM periods decreased compared to placebo (2.1 vs. 3.7, p<.01). Although the length of the first REM period was increased on high 5HTP dosages (43 vs. 18 min, p<.02), the time from onset of the first REM period to the second was unaltered.

The interpretation of the effects of 5HTP on sleep are complicated by such factors as dose, tolerance, and patient's behavioral response. Contrary to the hypothesis relating serotonin to sleep maintenance, total sleep decreased on 5HTP and contrary to the phasic-event suppression hypothesis, the REM density was not affected; however, REM density may not be the appropriate measurement of PGO spike density.

C. Biochemical: Cholera toxin is a non-reversible activator of adenylate cyclase from several tissues. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we used cholera toxin to study whether adenylate cyclase was linked to regulatory receptors of serotonergic neurons. The rate of 5-HT metabolism was taken as an index of neuronal activation and we measured this metabolism in brain areas of rats injected with cholera toxin into specific brain regions.

Cholera toxin was injected into the raphe nuclei or caudate nucleus and serotonin metabolism was studied by high pressure liquid chromatography.

When cholera toxin was injected into the raphe nuclei, the metabolism of serotonin was increased in the striatal regions on both sides of the brain. Increased metabolism was manifested by an increase in the concentration of 5-hydroxyindoleacetic acid (5HIAA)for more than 72 hours. The change was a direct result of an increased rate of formation of serotonin rather than an inability of the brain to dispose of the acid. Injection of the toxin or the vehicle for the toxin into the brain ventricles was without effect on the serotonin system. Injection of toxin into the caudate nucleus on one side of the brain resulted in increased levels of 5HIAA in the dorsal raphe nucleus and striatum on the opposite side of the brain. There were no detectable changes of 5HIAA in the medial raphe, whose axons do not project to the striatum. Activation of the dorsal raphe was apparently by a retrograde process. The basal levels of adenylate cyclase were enhanced on the injected side of brain; however, there was no change of adenylate cyclase activity on the contralateral side of brain even though 5HIAA levels increased. The rise of 5HIAA on the contralateral side of brain was prevented by a hemisection caudal to the injection site. Our results suggest that it is possible to biochemically activate the metabolism of brain serotonin for days using regional injections of cholera toxin. Activation is not necessarily associated with long term changes of adenylate cyclase activity. Moreover, it appears that both caudate nuclei may be innervated by a single cell body or by separate cell bodies in ready communication.

Many psychiatric disorders are associated with abnormal metabolism of the biogenic amines in brain. Adenylate cyclase can be activated by the biogenic amines. Colera toxin offers the opportunity for understanding the mechanisms for activating the cyclases of the amine containing neurons for prolonged periods.

The Habenula: Anatomical evidence suggests afferents to the raphe nuclei originating in the lateral habenula. The habenula nuclei have been proposed to play a unique role in funneling information from forebrain structures to the raphe. Supporting this hypothesis, recent electrophysiological experiments characterized neurons in this pathway as inhibiting the firing of raphe neurons. GABA has been postulated as the inhibitory transmitter. We have investigated in rats the short and long-term effects of habenula lesions on serotonin (5HT) turnover in the dorsal raphe and medial raphe and caudate nucleus, a dorsal raphe terminal area. In addition, GABA concentrations were determined in the dorsal raphe. Alteration in 5HIAA levels following lesions were used to estimate change in 5HT turnover. Both unilateral and bilateral habenula lesions produced statistically significant elevations in 5HIAA in the dorsal raphe when measured 16 hours and 7 days post lesion. There was no change in medial raphe or caudate nucleus 5HIAA. Dorsal raphe GABA concentration did not differ from control 7 days following biolateral habenula lesions. Although anatomical evidence suggests habenular projections to both dorsal and medial raphe, habenular lesions affect 5HT turnover only in the dorsal raphe. The lack of effect of habenular lesions on caudate nucleus 5HT turnover suggests that changes in cell body 5HT metabolism can occur without concomitant changes in terminal projections. These results support the presence of an inhibitory habenular raphe pathway but do not confirm GABA as the inhibitory transmitter.

Our finding of increased 5HT metabolism in the DRN following HBN lesions suggests that HBN-DRN afferents are inhibitory and complements previous findings of decreased 5HT neuronal firing in the DRN after HBN stimulation. The nature of the inhibitory input from the HBN is complex, possibly involving the depolarization of inhibition raphe interneurons by substance p-containing habenula-raphe afferents. Our results demonstrate that the habenula exerts physiological control of a specific raphe nucleus. Since both the DRN and MRN regulate different behaviors, the ability to selectively affect DRN metabolism via habenular manipulation should prove a useful tool in further studying the divergent function of the brainstem raphe nuclei.

III. The Catecholamines

A. Behavioral

AMPT (Alpha-Methyl-Para-Tyrosine): Several lines of evidence suggest that certain types of schizophrenia may be associated with excessive dopamine activity. Psychotic symptoms in schizophrenics may be intensified by methylphenidate, which increases functional dopamine activity, and by L-DOPA, which is converted to dopamine. Amphetamine-induced psychosis in volunteers and in "speed freaks" is thought to be mediated by dopamine. The clinical efficacies of neuroleptics probably depend upon blockade of dopamine receptors. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate limiting enzymatic step in dopamine synthesis. Although it should theoretically be clinically helpful in schizophrenics, two previous studies reported no effect when given alone. In a third study, however, Carlsson and his associates found that during treatment with AMPT, less thioridazine was required to treat schizophrenics. In this study, we report results of administering AMPT in a double-blind fashion to chronic schizophrenics who were concurrently treated with high doses of phenothiazines.

The subjects were 10 male chronic schizophrenics (age 19-35) who had been hospitalized for 2-15 years. Each received a constant dose of phenothiazines (average equivalent dose of chlorpromazine=800 mg/day). A four-week period of placebo administration preceded and followed by a three-week trial of AMPT (3 gm/day). Clinical effects were monitored by (a) the NIMH Nursing Rating Scale; (b) the BPRS administered by two psychiatrists weekly; and (c) the Comprehension Subscale of the WAIS before, during, and after AMPT administration.

As shown in the table, significant improvement was observed on the Comprehension Subscale ($p<.001$ compared with placebo periods) on AMPT. However, no improvement was noted in the schizophrenia items of the BPRS or the NIMH Nursing Rating Scale, except for one patient. Clinical impressions of three, nonblind psychiatrists suggested moderate but transient improvement during the first week of AMPT administration in 8 of 10 patients. Two patients developed symptoms on AMPT and one was withdrawn from the study. AMPT's effect on plasma levels of chlorpromazine (measured twice weekly) are presented in the table.

MEAN PLASMA CPZ (ng/ml)

Placebo	77.7	
AMPT	72.9	$p<0.01$
Placebo	109.0	

AMPT markedly increased prolactin secretion (see table).

MEAN PLASMA PROLACTIN (ng/ml)

Placebo	46.1	$p<0.002$
AMPT	111.6	$p<0.01$
Placebo	42.4	

MEAN (±SEM) SCORES ON FIVE CLINICAL OR PSYCHOLOGICAL VARIATIONS WITH AMPT

	Placebo	AMPT	Placebo	Analysis of Variance
Mean Psychosis Score (NIMH Inpatient Behavioral Rating Scale)	11.4±1.1	11.9±0.9	1.2±1.4	$X_r^2 = 4.20$,NS
Mean Schizophrenia Score (BPRS)	9.5±1.2	7.5±1.1	8.4±1.1	$X_r^2 = 5.85$, NS
Mean Comprehension Subtest Score (WIAS)	6.6±0.9	10.3±0.8	8.0±0.8	$X_r^2 = 11.56$ ($p<0.001$)
Mean Depression Score (NIMH Inpatient Behavioral Rating Scale)	7.0±0.5	8.0±0.4	7.1±0.5	$X_r^2 = 5.45$, NS
Mean Depression Score (BPRS)	5.9±1.3	4.8±1.2	4.8±1.3	$X_r^2 = 0.95$, NS

The results suggest that during a three-week trial with AMPT, an inhibitor of dopamine synthesis, subtle or no improvement in psychological functioning (judgment and comprehension) occurred in chronic schizophrenic patients on phenothiazines. Significant clinical improvement was not detected on the BPRS and NIMH Nursing Rating Scale.

In a second study, we administered AMPT (3 gm/day) for two-three months to seven chronic schizophrenic patients who were treated with thioridazine (800 mg/day). No major clinical benefits were documented in the second study, although serum prolactin appears to have been elevated, an indication of central dopaminergic blockade.

7-Hydroxy-Chlorpromazine: A number of biochemical, animal and human studies suggest that 7-hydroxy-chlorpromazine (7-OH CPZ) is an active metabolite of chlorpromazine (CPZ). This hypothesis was tested by administering 7-OH CPZ to four hospitalized chronic schizophrenic males (Research Diagnostic Criteria) using a double-blind crossover design.

Following a four-week placebo period, 7-OH CPZ was given for at least four weeks, three weeks of which were at 1000 mg/day. A second four-week placebo period was then followed by the administration of CPZ at the same dose and duration as 7-OH CPZ. Throughout the study, patients were evaluated twice/day with a nurse' rating scale and once/week with the BPRS. Serum prolactin levels were measured at least once/week. Data was evaluated by an analysis of variance and the Newman-Keul test.

Patients improved on 7-OH CPZ relative to placebo on the following NRS items: tension and uncooperativeness ($p<.01$); and blunted affect, hostility, excitement and arousal ($p<.05$). With regard to CPZ relative to placebo, improvement was demonstrated on tension and blunted affect on the NRS ($p<.05$). 7-OH CPZ was more effective than CPZ on uncooperativeness on the NRS ($p<.01$). On the BPRS, patients improved 7-OH CPZ relative to placebo on total score and emotional withdrawal ($p<.05$). Again, on the BPRS, CPZ was more effective than placebo on activation and anxiety-depression ($p<.05$). Finally, 7OH CPZ reduced emotional withdrawal more than CPZ ($p<.05$) as measured on the BPRS. Improvements on the NRS and the BPRS ranged from 25.5% to 50.9% for statistically significant items. Prolactin levels on 7-OH CPZ and CPZ were 33.1 ± 4.5 ng/ml and 31.1 ± 8.7 ng/ml, respectively. These were elevated by 119.2% and 84.0% over placebo levels of 15.1 ± 4.5 ng/ml and 16.9 ± 4.5 ng/ml, respectively ($p<.05$).

The above results clearly show that 7-OH CPZ is an active neuroleptic producing clinical improvement in chronic male schizophrenics. Moreover, it caused substantial increases in serum prolactin levels, a property of its metabolic precursor, CPZ, and numerous other antipsychotic dopamine blocking neuroleptics.

Propanolol: It has been reported that high doses of the β-adrenergic blocking agent propranolol can be therapeutic in schizophrenia. In an effort to confirm this finding, 11 young chronic schizophrenic patients have entered an ongoing double-blind crossover study of their clinical and metabolic response to this drug.

Subjects were patients on two research wards, who (1) were between age 18 and 45 years, (2) met Research Diagnostic Criteria for schizophrenia, (3) had had adequate trials of conventional neuroleptic treatment without achieving sufficient remission to enable living outside of a hospital setting, and (4) were able to consent or assent to participate in therapeutic trials. Patients were maintained on stable doses of neuroleptics throughout the trial. Treatment order, active-placebo or placebo-active, was determined randomly. Propranolol was given incrementally over a 5-wk period to a maximum of 1920 mg/24 hrs in two divided doses, unless the presence of side effects dictated a lower maximum. Patients were kept at their highest stable doses for 4 weeks and then tapered off propranolol over a further 4-wk period. Behavioral status was evaluated by the nursing staff who were blind to medication status using an expanded version of the Brief Psychiatric Rating Scale (BPRS). Behavioral ratings were analyzed as previously described.

Of the 11 patients entered in the protocol, 7 have completed a month of placebo and a month of stable dose of 1600 or 1920 mg of propranolol. One patient refused further participation after one week and a second patient had to be withdrawn because of postural hypotension. The results to date indicate the presence of modest changes in some ratings for each patient. No patient had a dramatic response, but the improvement in 2 patients seemed substantial to those who knew them.

Our clinical results thus far do not confirm earlier reports of marked benefit to treatment resistant patients, but the duration of treatment was longer in those trials. Further experience will be required to determine if there is a subgroup of patients who are responsive to treatment with propranolol. If such a group can be defined, it would be of great interest to discover the mechanism of action of propranolol in that group. Since propranolol is active both centrally and peripherally, and in addition has membrane stabilizing properties and antiserotonergic actions, there would be many possibilities.

IV. Physostigmine

Ten patients received oral physostigmine in varying degree in a controlled double-blind protocol attempting to replicate the earlier findings of Rosenthal that brief, but dramatic, clinical improvement could be observed during such treatment. None of the patients studied showed significant improvement. At this time, no further studies are contemplated.

V. Pineal Extract

Dr. Altschule reported in 1957 that injections of an aqueous extract of bovine pineal substance exerted a beneficial effect in chronic schizophrenia. A subsequent blind study on eight elderly, extremely chronic lobotomized schizophrenic women failed to show major therapeutic potential of the extract. We are testing the extract on a less chronically-ill population to see if there might be any merit to Altschule's earlier observations.

When the patients are clinically stable they are started blind on either a course of placebo or pineal extract. After 4-6 weeks on this medication, there is a crossover to the type material not already received. If the findings warrant, there is another shift of medication.

From the previous study done here using younger patients, it appears that there is a statistically significant but generally slight beneficial effect exerted by the extract on some patients. Only one patient can be said to have achieved a major remission associated with extract treatment. This finding has been confirmed in numerous crossover experiments.

A further three patients have been tried on pineal extract. Of the three, one responded with significant clinical improvement. Six months after completing the protocol, this patient was given a second trial of pineal extract vs. placebo injections and again showed significant clinical improvement coincident with extract administration.

The most pronounced responder had been tried on enteric coated pineal tablets. Although an open study, the results were sufffficiently encouraging to justify having more of these tablets made for further clinical trials, again in selected patients. The advantage of administering the pineal substance in this form is that dosage can be increased. The earlier studies with intramuscular preparations was handicapped by the inherent volume limitations imposed by that route of administration.

Two additional patients have been tried on enteric coated pineal powder tablets. One became markedly aroused and hostile; with the other there was no change. The one patient already shown experimentally to be a marked responder to the aqueous extract and thought in an open study to be responsive to the oral preparation went through a double-blind placebo substitution. The patient showed worsening of her psychosis (as judged by the nursing ratings) while on placebo and improvement when active medication was substituted. Further extensive clinical testing of pineal materials does not seem justified at this time. Efforts are being made using experimental animals to develop techniques for either predicting who the few pineal responsive patients might be or what might be a possible mechanism of action. Until a working hypothesis along these lines can be developed, it seems more practical to test clinically only those few patients who have a clinical profile quite similar to that of the most responsive patient.

We were unable to isolate a fraction of the pineal extract that would be significantly enriched with regard to inhibitory activity on the in vitro assay of indoleethylamine-N-methyl transferase. Further study with pineal and liver extracts indicated that the inhibition obtained from extracts of these tissues was quite non-specific.

VI. Lithium Carbonate with Other Neuroleptic Drugs

There is good documentation in the literature indicating that chlorpromazine is superior to lithium in acute schizophrenic illness. The superiority of chlorpromazine in controlling extreme mania also appears to be established. Publications bearing on the use of lithium in schizo-affective illness are less unanimous in their conclusions. All of the studies so far reported do not indicate a superiority for the use of lithium over chlorpromazine in patients diagnosed as schizo-affective. There are no studies of merit using both medications simultaneously. It is our personal experience that many clinicians are using lithium carbonate in combination with major tranquilizers in the treatment of schizophrenic and schizo-affective patients, despite the fact that there are no controlled experiments in the literature bearing on the efficacy of this combination.

This study has been expanded and is taking place mainly outside the William A. White Research wards. This has been done for a number of reasons, among them our interest in being able to study a greater number of patients on low-risk protocols such as this one. After several month's work, three wards in other services have been trained in the use of the rating instruments. In addition, one of the wards is in John Howard Division. This setting offers an excellent opportunity to study the effects of lithium ion on various aspects of aggression. The studies have been completed, and we are in the process of analyzing the data and preparing the manuscript for publication.

Lithium has been extraordinarily successful in the treatment of manic-depression. To compute the economic impact of that success on the United States, an estimate of the cost of care for manic-depression before lithium was introduced was compared with cost estimates after lithium. Economic gains in production were also calculated. Assumptions and exclusions err on the conservative side so that estimates, if inaccurate, are low. The use of lithium as a treatment for manic-depression has saved $2.88 billion in ten years and resulted in a $1.28 billion gain in production, or a conservative total of over $4 billion.

Computing lithium's impact on the cost of manic-depression required examining the cost of treating manic-depressive patients before and after the introduction of lithium. The task was not straightforward. It required (1) quantifying human life--we chose to do this through income, and (2) making a number of assumptions. Since little or no information was available on the number of manic-depressive patients in the United States, the number in treatment, their average length of stay in a hospital, and their basic pattern of treatment (including number of hospitalizations, outpatient visits and loss of worktime), the computations themselves rested on little hard data.

Our estimated impact of lithium on the cost of psychiatric care is impressive. Just as good nutrition and penicillin began to empty psychiatric wards of the victims of pellegra and syphillis and drastically reduce the costs of these diseases, lithium decreased the cost of manic-depression.

Lithium has a remarkable ability to ameliorate the mood swings of a manic-depressive. It is readily available and inexpensive. Serious side effects are uncommon and most side effects are reversible. Furthermore, a response to lithium is widely thought to be diagnostic; many people who do respond to lithium would not previously have been diagnosed as manic-depressive and, perhaps, would not have received proper treatment.

Clearly, lithium has saved money. We estimate that in 10 years, from 1969-1979, America saved at least $2.88 billion in medical costs and gained $1.28 billion in production, a conservative total of around $4 billion as a consequence of the use of lithium to treat manic-depression. While our estimates are speculative and rest on limited data, they are--if inaccurate--extremely conservative. More solid calculations will become possible when more reliable information on numbers and diagnoses of people treated for psychiatric disorders are generated.

VII. GABA

A. Clinical

Baclofen (Lioresal): In the spring of 1975, a brief clinical report appeared in Lancet stating that 13 treatment resistant chronic schizophrenics were found to improve and require less conventional neuroleptic medication when placed on Baclofen. Since Baclofen, then thought to be a GABA agonist but now thought to be an antagonist of substance P, is a compound in no way similar to currently used neuroleptic agents we have conducted a clinical trial.

In an attempt to replicate claims of baclofen's efficacy in treating schizophrenia baclofen was given to young chronic schizophrenic patients and plasma prolactin was monitored to study the mechanism of action of baclofen. Seven hospitalized male chronic schizophrenic patients were stabilized on clinically suboptimal doses of conventional neuroleptics (equivalent to 800 mg/day of chlorpromazine). Experience with these patients revealed that these doses were necessary for participation in the protocol and that larger doses resulted in less symptoms but not to the point that patients could function outside of the hospital. Baclofen was then added in doses of 80 to 120 mg/day for one month in a double-blind, crossover design. Twice weekly, fasting bloods were drawn 11 hours after the last neuroleptic and baclofen dose (into heparinized tubes which were then placed on ice). Plasma was removed and samples stored at -70 C until radioimmunoassay by the double antibody technique. The prolactin values during each period were stable for each patient as reflected by an intraclass correlation of .93 for the neuroleptic period and .87 for the baclofen period. For each patient, biweekly prolactins were meaned separately for the placebo and active periods and then compared by paired t-test.

No increase in plasma prolactin was found with the addition of baclofen (37.08 vs. 37.57 ng/ml, paired t-test, NS) to patients given sub-optimal doses of neuroleptics. There was no evident difference in response on 2 dose levels. Additionally, the patients did not clinically improve nor did they demonstrate change on rating scales.

Failure of baclofen to elicit an increase in plasma prolactin in these patients might be explained in several ways. Either the 80 to 120 mg/day dose (approximately 1 to 1.5 mg/ky) or one-month duration might be insufficient. In the rat, Meltzer and Fang found that only doses of 25 mg/kg and above produced increases in prolactin. Ravitz and Moore found that 5 and 10 mg/kg doses of baclofen slightly reduced prolactin, while 20 mg/kg markedly increased serum prolactin in the rat. They felt that the 20 mg/kg dose was anaesthetizing in the rat and the rise in prolactin was a non-specific stress-induced effect.

It is possible that prolactin in the patients was already maximally stimulated by the presence of neuroleptics and no further change would be expected by addition of baclofen. However, five of these seven patients, while similarly stabilized on neuroleptics (chlorpromazine equivalent to 800 mg/day), showed a dramatic rise in prolactin from 51 to 117 ng/ml (5=3.32, $p<.05$) when treated with three gms/day of AMPT.

Although baclofen has been used successfully to treat multiple sclerosis and other neurological disorders, its mechanism of action is still open to question. This study in humans demonstrates no alteration in dopamine metabolism as reflected by plasma prolactin concentrations.

B. Animal

Imidazoleacetic Acid: Chronic administration of neuroleptic drugs to animals results in a syndrome of behavioral hypersensitivity to dopaminergic agonists. It is thought that this is a manifestation of the development of postsynaptic supersensitivity to dopamine, induced by the dopamine-receptor blocking activity of these drugs. The phenomenon of postsynaptic denervation supersensitivity has long been known to be nonspecific in regard to particular neurotransmitters; this would suggest that chronic treatment of animals with dopamine agonists would also induce alterations in behavioral sensitivity to any other neurotransmitters for which receptors are located on the same neurons as dopamine receptors. There are gamma-aminobutyric acid (GABA) receptors in the striatum, and these seem to be intimately associated with dopamine-receptive neurons. It was therefore decided to test the possibility that alterations in the behavioral sensitivity to GABA agonists accompany the behavioral hypersensitivity to dopamine that is induced by chronic treatment with the dopamine-blocking neuroleptic haloperidol. It was found that the ability of the GABA receptor agonist imidazole-4-acetic acid (IMA) to reduce motor activity was lessened in animals that had been given haloperidol chronically added to the drinking water. When the time course of the behavior of the animals was examined, it was found that the activity of the control animals was high when first placed in a novel environment, but habituated gradually over the course of testing. Animals treated chronically with haloperidol, or injected with IMA, showed relatively small changes in total activity but essentially the same pattern of habituation. On the other hand, animals treated chronically with haloperidol and also injected with IMA showed almost a complete absence of habituation, in that their activity remained at or near its initial levels for the entire duration of a two-hour testing session. Acute injections of haloperidol had no such effect, and were simply additive with IMA in terms of decreasing behavioral activity. It is suggested that this alteration in responsivity to IMA, and presumably to central nervous system plays a role in the therapeutic response of schizophrenic patients to haloperidol.

VIII. Thyrotropin Releasing Hormone in Chronic Schizophrenia

Intravenous administration of synthetic thyrotropin releasing hormone (TRH) has been reported to lead to a prompt amelioration of schizophrenic symptoms in a pilot study involving four female patients. Drayson gave TRH to three patients with cyclical psychoses and failed to observe any clinical change. We gave TRH to three treatment resistant chronic schizophrenic males in an inpatient research setting in which all subjects were drug free for at least a month prior to study. Synthetic TRH, 600 g (Abbott 38579) or matching placebo, was administered by slow intravenous injection at 8 a.m. for five days followed by a two-day holiday and then crossover to the treatment not given. Order of treatment was random; neither the person giving the injection nor any of the clinical staff was aware of the nature of the substance being given. The patient's clinical status was estimated by the nurses' rating scale as well as by clinical observation by the resident psychiatrist. Particular attention was paid to each patient the first day of each series of injections because of the reported rapid onset of clinical change. Statistical comparison of active TRH vs. placebo was by t-test of the means for appropriate questions for the five days of each treatment period. The results are summarized in the Table.

EFFECTS OF SYNTHETIC TRH ADMINISTRATION ON THE SYMPTOMS OF SCHIZOPHRENIA IN THREE CHRONIC SCHIZOPHRENIC PATIENTS. #: t-TEST

Patient # Treatment	Psychosis	Depression	Anxiety	Hallucinations & Delusions
Placebo	3.20±.09	1.53±.16	2.80±.15	1.35±.62
1) TRH	2.84±.25	3.18±.25	3.18±.22	2.20±.50
p	NS	.05	NS	NS
Placebo	3.47±.15	1.55±.30	2.68±.16	4.55±.28
2) TRH	3.23±.13	1.23±.20	3.10±.21	4.23±.38
p	NS	NS	NS	NS
Placebo	3.34±.12	2.28±.12	2.93±.24	3.80±.19
3) TRH	3.56±.14	3.53±.30	3.75±.12	5.43±.37
p	NS	.005	.01	.005

The table shows that two of the three patients showed some worsening of their clinical condition, particularly patient 3. It is especially interesting to note that the depression score for these two patients were significantly greater during the week on TRH as compared to the week on placebo. This finding stands in contrast to earlier reports suggesting that TRH has antidepressant activity, and certainly TRH does not appear to be a useful drug for schizophrenia.

IX. Formation of Tryptolines (Tetrahydro- -Carbolines) by Human Brain and Platelets

We have examined the products formed from tryptamine, N-methyltryptamine, and 5-hydroxytryptamine by an enzymatic preparation from either human brain or platelets, using 5-MTHF as a cofactor. In each case, we have demonstrated by TLC, co-crystallation, and gas chromatography/mass spectrometry that the reaction involves the addition of a carbon unit to the side-chain nitrogen, with subsequent cyclization to yield a tricyclic, tryptoline derivative. These compounds have been found in plants, but have not previously been associated with mammalian systems. For both enzymatic preparations, the optimal pH is 6.5 and phosphate buffers of increasing strength enhance the reaction. For the brain enzyme, methylcobalamine, flavin adenine dinucleotide, and ascorbic acid each enhance the reaction, while cupric ion inhibits it. We have also done preliminary investigations on platelet enzyme activities in normal volunteers. We found marked diurnal changes and wide variability among individuals; however, male enzymatic activities were significantly lower than those for females. These studies demonstrate that 5-MTHF does not act as a methyl donor, as previously believed.

We have examined the properties and distribution of the enzymatic reaction in the human brain, using 5-MTHF as cofactor. With indoleamines, activity increases with either hydroxy or methyl substitution at the 5-position of the aromatic ring and decreases with the addition of methyl groups to the side-chain nitrogen. Both tryptopline and 5-hydroxytryptoline are substrates but 1-methyltryptoline is not. The enzyme has a similar Km for tryptamine and N-methyltryptamine and is inhibited by Cu^{++}, Ca^{++} and dithiothreitol. The enzymatic activity is unevenly distributed in the brain, with the highest activity in the hypothalamus and the lowest activity in the thalamus.

Acute ip injections of 5-hydroxytryptoline (5-HTLN) killed mice and rats between 12 and 48 hours post-injection with an LD_{50} dose of 320 to 350 mg/kg. Two weeks of daily injections of 200 mg/kg or 100 mg/kg 5-HTLN resulted in respective mortalities of 100% and 50%; surviving mice showed no changes in weight or gross appearance of brain, liver, lung, heart, spleen, kidney or adrenal. Neither acute nor chronic injections affected body weight or temperature except immediately before death where both dropped markedly.

In mice, one hour pretreatment with 200 mg/kg 5-HTLN ip, increased secobarbitol-induced "sleep time" (50 mg/kg, ip). At these doses, neither drug, alone, is lethal; yet, 50% of the animals treated with both drugs died 24-36 hours later. Intraventricularly, 10 g of 5HTLN to rats decreased motor activity for 25 minutes following the injection.

Acute application of 150-300 g of 5-HTLN to the isolated rat fundus strip contracts the muscle in a manner similar to 2-4 ng of 5-HT. When added to the superfusion medium, 1.0 g of 5-HTLN causes a tonic increase in muscle tone without blocking 5-HT-induced contractions. Methysergide blocks these 5-HTLN effects. Preliminary studies suggest that 5-HTLN may act on serotonergic mechanism in the brain.

A case of folate-responsive psychosis which was associated with a defect in $N^{5,10}$-methylenetetrahydrofolate reductase (methylene reductase) suggested the need to examine whether abnormally low activity of this enzyme might be of etiological significance in schizophrenia. We found that there was no significant difference in the platelet methylene reductase activity of chronic schizophrenics, compared to either hospitalized or nonhospitalized, age-matched control subjects (see table). Although it is possible that a larger survey might reveal a subpopulation of schizophrenics who are characterized by abnormal methylene reductase activity, this study suggests that chronic schizophrenia is not generally associated with such changes.

Comparison of Platelet Methylene Reductase Activity in Schizophrenic Patients and Control Subjects

	N	Age (Mean±SEM)	Methylene Reductase Activity (Mean±SEM)*	Range*	r**
Normal Controls	15	28.7±0.94	0.86±0.12	0.15±1.36	-0.31
Hospitalized Controls	16	27.7±1.25	0.72±0.13	0.15±1.36	-0.32
Schizophrenics	18	28.1±1.19	1.17±0.18	0.05±2.28	- .35
All Subjects	49	28.1±0.65	0.93±0.09	0.01±2.28	- .31+

*Expressed as nmole/hr/mg protein; **Correlation of age to methylene reductase activity; +$p<0.05$

X. Sleep

The sleep of 13 drug free chronic male schizophrenics was studied during a one-month trial on chlorpromazine (CPZ) and compared with one-month placebo periods. Recordings were made only after subjects had been on the drug or placebo at least three weeks. Sleep latency and awake time was significantly less on CPZ while Stage II, Delta Sleep, Delta %, Non-REM activity, and REM Density were significantly increased. There were no significant changes in REM Latency, REM time, REM %, Stage II %, and Non-REM %.

We investigated whether schizophrenics have a normal REM rebound following REM deprivation. In addition, since Cartwright et al. previously suggested that "good REM compensators" tend to be field independent, we related a measure of field independence (the portable rod and frame test) to degree of compensation.

Eight actively ill schizophrenic patients (E) (5F, 3M, mean age = 25) and eight non-psychotic control patients (C) (5F, 3M, mean age = 26) were partially REM deprived by the awakening method for two nights. Sleep on each of five Recovery nights (R_1-5) was compared with that of Baseline Sleep. All patients were unmedicated except for a single control patient who received chlorpromazine.

Mean Baseline Sleep patterns (min) were similar in E and C patient groups:

	Total Sleep	REM Sleep	REM Latency	REM %	Stage III + IV
E	378	93	72	25.0	37
C	390	99	78	25.5	32

Mean loss of REM was 71.8% for E and 72.8% for C. Controls had to be awakened significantly more frequently than E (35 awakenings compared with 28, $p<.05$).

On R_1 mean change in REM compared with Baseline was +38 min for C and -2 min for E ($p<.01$); on R_2 it was +17 for C and -10 for E ($p<.05$). Mean change in REM during all Five Recovery nights was +14.5 min for C and +1 for E ($p<.05$).

A two-way multivariate analysis of variance--one factor being patient groups, the other being a repeated measure, linear trend analysis--revealed that C had significantly different sleep patterns than did E during Recovery ($p<.03$, $F=2.45$). The major source of difference was in REM sleep recovery ($p<.006$, $F=8.06$) but, surprisingly, Stage III+IV was also a significant contributor ($p<.02$, $F=6.13$). E tended to have elevated Stage III+IV on the first two Recovery nights and low and normal amounts on the last two except on the last Recovery night when Stage III+IV tended to be elevated, though not significantly.

REM rebounds during Recovery were correlated with increased Total Sleep ($r=.52$, $p<.01$). Nevertheless, after removing the effect of change in Total Sleep during Recovery by analysis of co-variance, the controls still had the normal pattern of REM rebound and the actively ill schizophrenic patients did not, i.e., the two groups differed in rate of REM decline over the five Recovery nights ($p<.03$).

Among four E and four C patients tested, there was a significant correlation between degree of REM rebound and field independence ($p<.05$, rho=.79). Mean change in REM during Recovery was 27 min for three field independent patients and -1.5 min among five field dependent subjects ($p=.036$, Mann-Whitney U-test).

These results suggest that actively ill schizophrenics differ from other psychiatric patients in failing to have a normal REM rebound. This difference was not attributable to differences in age, sex, baseline sleep, anxiety, or change in Total Sleep time during Recovery. REM compensation may also be correlated with field independence, though further research needs to be done to substantiate this finding before any conclusions may be drawn.

XI. Evoked Potentials and EEG

We studied the evoked potentials of 21 schizophrenics and 21 controls. They were matched for age and exposed to a 10-minute sequence of frequent and infrequent auditory stimuli. The amplitude of P_3, a response component of the evoked responses to infrequent stimuli, was much larger in the control group ($p<.0003$). Using a P_3 amplitude of 3.20 V in the first 2.5 minutes as a dividing point, only 7 of the 42 subjects were misclassified. This amplitude had a significant correlation of 0.48 with the patient's age at first hospitalization. The group P_2 amplitude to frequent stimuli was also much larger in the control group ($p<.003$). This amplitude has a significant correlation of -.41 with medication dosage in terms of equivalent amounts of chlorpromazine. Behavioral scales failed to correlate significantly with evoked response measures. Evoked response latencies did not distinguish the two groups.

A study is underway to develop a means of demonstrating whether or not schizophrenic patients have an unusual lateralization of brain functioning. Visual processing of verbal and non-verbal tasks is being tested to show if patients have recognizable differences from normal subjects. The tasks involve the use of a projection tachiostoscope and are being presented to normal subjects to establish normal patterns of response. Patients are being tested also, but inconsistent results have developed from limited trials. Should the theory behind this study prove workable, it could be applied to tests of various drug effects, or used to monitor the progress of treatment of patients in addition to providing information about a possible aspect of mental dysfunction.

XII. Brain Dopamine-β-Hydroxylase

Stein and Wise (1971) postulated that there might be a deficit in dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, in schizophrenic patients.

Wise, Baden and Stein (1974) tested their hypothesis by collecting the brains of 18 chronic schizophrenics who had died in a state hospital and 12 persons without a history of psychiatric illness dying suddenly of heart attacks or accidents. The schizophrenics had decreased DBH activity ranging from 49 in the hippocampus, 58 in the diencephalon and 70 percent in pons-medulla. All these differences were statistically significant at $p<.05$. Because of these dramatic differences in the predicted direction, Wise et al. made a number of attempts to control for artifacts. After considering all these sources of artifacts, the large differences between schizophrenic and normal DBH activity remained.

We attempted to replicate their study. Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at Saint Elizabeths Hospital. Brain specimens from four chronic schizophrenics and nine controls were obtained by similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members, and hospital and police records. The control brains were obtained from periods without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The schizophrenics (7 males; 2 females) had a mean age of 49.2 ± 6.3. Four died suddenly after traumatic suicides (in our experience, suicide is common among chronic schizophrenics), three from cardiac arrests, one from pulmonary aspiration, and one suddenly from pulmonary edema. The controls (all male) had a mean age of 42.3 ± 4.5. Six died suddenly of trauma and three from cardiac arrests.

The periaqueductal pons-mesencephalon, hypothalamus and hippocampus were dissected out at autopsy and immediately placed on dry ice and subsequently stored at $-80^{\circ}C$ for up to one year. Wise et al. (1974) found DBH activity stable for one year at $-15^{\circ}C$. There was no difference in mean storage time for the schizophrenics and controls. All DBH assays were performed by a staff member unaware of whether the samples were from schizophrenics or controls.

There was no significant difference in DBH activity in any area between the two groups. While the differences were not statistically different, DBH activity mean for the schizophrenic groups ranged between 77 and 89 percent of controls. Although seven of nine of our patients were taking phenothiazines at the time of death, Wise et al. (1974) indicate that this is probably not a cause of their DBH differences. Rats who had been given chlorpromazine (20 mg/kg 24 hour) for 12 weeks had a small increase in DBH activity. Although the patients in our study were taking a number of non-neuroleptic drugs, there was significant ($p<0.05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus ($r=-.60$) and pons ($r=-.65$). This could mean that in the brains of schizophrenics, neuroleptics do decrease DBH activity, or possibly that there is a negative correlation between clinical pathology (as determined by the need for higher dosages) and DBH activity.

Wise et al. (1974) also attempted to determine the effects of post-mortem intervals on brain DBH activity (after a person dies, it may take several hours for that person to reach the morgue). Simulating this situation, rats that were killed and allowed to remain three hours at room temperature prior to assay had DBH activity reduced by 27%. In a similar experiment performed in our Laboratory over a six-hour period, there was 15% decrease in activity. Since Wise et al. (1974) human brain post-mortem intervals were "several hours" and ours a mean of 4.2 for the controls and 3.0 hours for the schizophrenics, this does not seem to be an important factor, except possibly for one control who was not brought to the morgue for 27 hours. Since he had the lowest DBH activity of any of the subjects, the DBH activities were examined for the controls with and without this subject's values. The exclusion of this value did not change the failure to reach statistical significance. Two subjects with death-to-morgue times of seven and 11 hours had DBH activities about their group means. One subject with a 3.5 hour time lag was low. All other subjects had intervals of about one hour.

Wise et al. (1974) also compared the effects on brain DBH activity of the time from which the subject reached the morgue to the time of autopsy. They did this by storage of rat brains at 4°C for up to three days and found a decrease in DBH activity of 18%. Their subjects' brains, however, ranged from one day to eight days at this temperature. Because of this, they matched time intervals for controls and schizophrenics and still found the DBH activity of the schizophrenics lower. Our controls were in the morgue for a mean of $9.7+1.9$ hours, while the patients were there for $35.6+7.9$ hours, which is statistically significant ($t=2.73$, $p<.02$). Furthermore, there are significant negative correlations between the time in the morgue to autopsy and DBH activity in the hippocampus ($r=-.53$, $p<.05$) and hypothalamus ($r=-.49$, $p<.05$) while that for the pons ($r=-.46$) was not significant. This suggests that the uneven distribution between the two groups in morgue time may be responsible for the small non-significant differences in DBH activity in our samples. Since the Wise, Baden and Stein brains came from two sources, it is possible some of their group differences may be due to different storage techniques.

We measured DBH activity both under maximal conditions as well as under non-maximal conditions, but did not find significant differences among patient groups and normal controls by enzyme procedure.

Differences in population characteristics such as race and sex is suggested by the trend in blacks to have lower DBH activities and females to have higher activities than males. The relationship of age to DBH enzyme activity is not known but may also be a contributing factor and should be further explored.

Previous reports have not determined DBH activity in various subtypes of schizophrenia. Although we found no significant differences among the subtypes and normals, the chronic undifferentiated group with paranoid features had a trend toward lowered DBH activity ($p<.2$ and $p<.13$). Further investigation of this subtype appears warrented.

One way analysis of variance shows no significant difference between control and schizophrenic subpopulations. The chronic undifferentiated schizophrenics with paranoid features had a somewhat lower mean DBH than the other patient groups or normal controls, although this does not reach statistical significance. The normal control group had a mean DBH of 37.28 ± 26.00.

In summary, in our investigations, we have been unable to confirm the findings of low plasma DBH activity in chronic schizophrenic patients or the association of low DBH and MAO activity in pedigrees of schizophrenic probands.

XIII. Dopamine Sensitive Adenylyl Cyclase

Striatal adenylyl cyclase activity from autopsied human brain is selectively stimulated by low concentrations of dopamine. Under the experimental conditions used, dopamine was about three times more potent than norepinephrine. Histamine and serotonin were ineffective. This stimulation by dopamine was competitively inhibited by haloperidol. There was no difference between nine of the autopsied brains (head of caudate) of the control subjects without a psychiatric history and seven patients with chronic schizophrenia.

IX. Biogenic Amines, Their Precursor Amino Acids and Their Metabolites

The role of biogenic amines in mental illness may include amines other than serotonin and catecholamines. In the present state of our understanding, little information is available on these amines. To bridge this gap, we have undertaken the task to develop comprehensive and sensitive mass fragmentographic methods to measure many of the known biogenic amines, their amino acid precursors and their metabolites.

Our initial goal is to apply the method developed to measure the concentrations of the various amines and their derivatives in plasma, cerebrospinal fluid (CSF) and brain tissues. After establishing the normal ranges of these substances in biological materials, we hope to carry out several clinical and pharmacological studies in order to probe further the possible role of biogenic amines in mental illness.

The principal technique employed is mass fragmentography. To enhance the specificity of the method, selective gas chromatographic columns are used. These columns were chosen after extensive preliminary trials with various stationary phases and with different lengths of columns.

The biogenic amines, their amino acid precursors and their metabolites are partially separated and concentrated by either extraction into organic solvents or by thin layer or column chromatography. They are next dried down, derivatized to their appropriate pentafluoropropionate or trifluoroacetate derivatives and then injected into the GC column.

To correct for variations in processing the samples, internal deuterated isomers of the compounds of interest were synthesized, run through the whole procedure and then used to correct for any inconsistency.

The metabolites derived from tyramine, octopamine, dopamine and norepinephrine were extensively studied in the rat brain, lumbar and ventricular CSF. Mass fragmentography was carried out on the methyl ester/pentafluoroproprionate (ME/PFP) derivatives of the acidic and the PFP derivatives of the alcoholic metabolites, employing an 8 ft. 3% SE 52 column.

In nine patients with ventricular-atrial shunts (5 males, 4 females, age infancy to 65), ventricular metabolite concentrations (ng/ml, mean ± S.E.M.) were: p-hydroxyphenylacetic acid (22.4±3.6), p-hydroxymandelic acid (7.1±1), vanillylmandelic acid (3.1±0.3), dihydroxyphenylacetic acid (9.7±1.6), homovanillic acid (154±14.4), free 3-methoxy-4-hydroxyphenylglycol (MHPG) (11.7±1), conjugated MHPG (4.6±1.3), free p-hydroxyphenylethanol (PHPE) (12.3±1.7) conjugated PHPE (less than 2 ng/ml). The levels of the acidic metabolites in the lumbar CSF were all significantly lower than those in the ventricular CSF. The alcoholic metabolites on the other hand were not significantly different. P-hydroxyphenylglycol was also semi-quantitatively measured and found to be present almost completely in the free form.

Lumbar cerebrospinal fluid (CSF) of schizophrenic patients was studied.

Treatment with oral probenecid (100 mg/kg) increased the concentrations of p-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic and homovanillic acid by over three-folds compared to the baseline levels. The concentration of p-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency towards being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

P-hydroxyphenylacetic, p-hydroxymandelic, homovanillic, 3,4-dihydroxyphenylacetic, vanilmandelic acids and 3-methoxy-4-hydroxyphenylglycol were measured in control, acutely treated and ethanol-dependent rats.

Ethanol did not cause a shift from an oxidative to a reductive pathway in the metabolism of amines as is well established in the periphery. Ethanol was found to have no effect on the transport of the acidic metabolites across the blood brain barrier.

Metabolites derived from dopamine and norepinephrine were significantly elevated in both acutely and chronically treated rats while blood ethanol levels were high. During withdrawal (no ethanol was present in blood) only norepinephrine metabolites remained elevated in rats rendered dependent on ethanol.

The concentration of VMA and MHPG in whole rat brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8 percent. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 hr-1, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norepinephrine metabolism in brain.

The concentration of the free, glucuronide and the sulfate conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in human plasma, cerebrospinal fluid (CSF) and brain by mass fragmentography.

All three forms of MHPG were detected in the media analyzed. Free MHPG was found to be the predominant form in both the brain and the CSF. The sulfate conjugate of MHPG constitutes about 15% of the total MHPG in the CSF while in the brain the percentage varies between 30% in the hypothalamus and cortex and 80% in the substantia nigra.

The concentration of the glucuronide conjugate of MHPG measured in the brain and CSF represents about 5% of the total MHPG concentration.

In the plasma free MHPG and its glucronide and sulfate conjugates are present in about equal concentrations.

The relative concentrations of the three forms of MHPG measured in plasma, CSF and brain were compared with their concentrations in the urine from previously published results. From this comparison the diagnostic significance of each of the three forms of MHPG in the clinical assessment of central norepinephrine metabolism is discussed.

Human plasma concentrations of the major acidic metabolites of m- and p-tyramine, octopamine, dopamine, epinephrine and norepinephrine was measured. The free and conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were also measured. The means and ranges of concentrations of the acid metabolites were comparable except for m-hydroxyphenylacetic acid. Free, sulfate, and glucuronide conjugated MHPG were also found in about equal concentrations.

Vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in rat brain. The concentration of VMA and MPHG in whole brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8%. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of elimination of VMA and MHPG is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA is 1.4 and 0.38 h^{-1}, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norpinephrine metabolism in brain.

In a collaborative study with Dr. Norton Neff of the Laboratory of Preclinical Psychopharmacology, dopamine metabolism was studied in various regions of rat brain following the decline of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) from brain after treatment with pargyline, or from the accumulation of the acids after treatment with probenecid. The decline of DOPAC and HVA after pargyline treatment appeared exponential in all regions of brain studied with half-lives of about 13 min for HVA and 6.5 min for DOPAC. DOPAC was the major metabolite of dopamine, with various brain regions producing between 2 to 5 times more DOPAC than HVA. HVA accumulated after treatment with probenecid but the accumulation in one hour did not account for all of the HVA apparently eliminated from brain. DOPAC accumulated in some regions of brain (medulla, hypothalamus, and midbrain) and not in others (cerebellum, cortex, striatum, and hippocampus) after probenecid treatment. We conclude that dopamine metabolism is not uniform in brain and that the accumulation of DOPAC and HVA in brain after probenecid treatment only accounts for a minor action of the dopamine formed in brain.

Also in collaboration with the Laboratory of Preclinical Pharmacology a study was undertaken to measure phenylalanine hydroxylase activity *in vivo* by measuring ^{18}O-tyrosine in plasma after exposing rats to ^{18}O. We were able to show that ^{18}O is incorporated in tyrosine *in vivo* and that the presence of ^{18}O-tyrosine in plasma is a relative measure of phenylalanine hydroxylase activity. Treatment with p-chlorophenylalanine decreases the ^{18}O incorporation into tyrosine. Because of the simplicity of the procedure and its easy adaptability to human studies, the incorporation of ^{18}O into endogenous constituents might serve as a useful diagnostic procedure for some metabolic disorders, for example, phenylketonuria. These results also indicate that labelling with ^{18}O cannot be used to measure the turnover rate of brain catecholamines as previously proposed.

Sympathetic Ganglion: We also studied dopamine metabolism and function in the rat superior cervical ganglion. Trans-synaptic modulation of dopamine metabolism in rat superior cervical ganglion was evaluated by contrasting normal and surgically decentralized ganglions. Dopamine metabolism was assessed from the decline of DOPAC after administering pargyline and from the concentration of dopamine and DOPAC after treatment with drugs. The concentrations of dopamine, HVA and DOPAC were about 19, 2.7 and 51 pmol/ ganglion, respectively. DOPAC was the major metabolite of dopamine and its calculated rate of formation was 5.3 pmol/ganglion/min, which is approximately 300 nmol/g/hr. This is about 15 times faster than the rate of formation of dopamine reported for striatum. The rapid formation of dopamine in ganglion was supported by the finding of a rapid decline of dopamine after ℓ-methyl-tyrosine treatment. In the same rat decentralization of the ganglion reduced the rate of formation of DOPAC, but increased DOPAC formation above normal in the intact sham decentralized ganglion suggesting that a feedback system may modulate dopamine metabolism. Treatment with carbachol alone or in combination with atropine of hexamethonium revealed that dopamine metabolism is modulated by nicotinic and muscarinic mechanisms. HVA and DOPAC accumulated in ganglion after treatment with probenecid indicating that an acid transport system is probably responsible for removing these materials from the ganglion. The similarities between the dopaminergic system of the ganglion and of the brain and the rapid rate of dopamine metabolism in the ganglion supports the notion that dopamine might serve as a transmitter or modulator of synaptic events with the ganglion.

During the last 12 months, our work on the sympathetic ganglion was aimed towards achieving two goals: the first was to gain more knowledge on how preganglionic muscarinic and nicotinic cholinergic inputs affect the small intensely fluorescence (SIF) cells with regard to DA turnover and metabolism. The second goal was to characterize pharmacologically the catecholamine receptors present in the sympathetic ganglion and to determine how these receptors regulate catecholamine turnover and synthesis. The ganglion studies were: superior cervical (SCG) and celiac (CG).

The SIF cells were found to be mostly stimulated by muscarinic preganglionic nerves. For example, decentralization lowers DA turnover as reflected by a significant decrease in DOPAC concentration in the SCG. Administration of carbacol or oxotremorine (muscarinic agonists) increased DOPAC while atropine decreased DOPAC in both SCG and CG. Hexamethonium or arecholine (nicotinic agonists) have very little effects on DOPAC concentrations. However, nicotinic receptors appear to have some inhibitory effects on the muscarinic stimulation of the SIF cells.

The concentrations of DA, NE and DOPAC in the ganglia were critically evaluated after treatment with the following basic drugs: reserpine, haloperidol, apomorphine and phenyoxbenzamine.

Reserpine significantly decreased NE and increased DOPAC, but had no effect on DA concentrations in the sympathetic ganglia.

Apomorphine, contrary to what we expected, had no effect on catecholamine and slightly increased DOPAC (we expected a decrease). Pretreatment with apomorphine, however, antagonizes the effects of reserpine, thus indicating that reserpine and apomorphine exert their influences via a common receptor.

Haloperidol increased DOPAC in the CG but not in the SCG. It had no effect on the NE and DA concentrations. These results suggest some differences between the DA receptors in the SCG and the CG.

Phenoxybenzamine had no effect on DA, NE, or DOPAC in the ganglia.

XV. N,N-Dimethyltryptamine (DMT) and Phenylethylamine (PEA)

A. Behavioral

Two separate drug-induced behaviors in the rat that satisfy criteria which must be met for animal models of schizophrenia have been described. N,N-Dimethyltryptamine (DMT) and β-Phenylethylamine (PEA) were the two drugs studied because evidence suggests that they are viable candidates in the pathogenesis of schizophrenia. Both are endogenous compounds, found in human tissue. Both may be differentially elevated in psychiatric patients. When given to man, DMT produces psychological disturbance and amphetamine (almost structurally identical to PEA) can produce a syndrome resembling paranoid psychosis. Instead of trying to find an animal equivalent of schizophrenia, we studied the behavioral effects of these compounds in rats and applied to these behaviors criteria derived from the clinical condition of schizophrenia. Both DMT and PEA produced dose response disruption of a well-trained conditioned avoidance response in the shuttlebox and PEA, with monoamine oxidase inhibition by pargyline, induced dose response stereotypy, associated with increased motor activity. The criteria we tested were alterations of the drug induced behaviors by acute or chronic neuroleptic pretreatment and tolerance development. Neuroleptics, which are efficacious in the treatment of schizophrenia, reduced DMT disruption and PEA stereotypy in a dose response relationship. In the case of DMT, disruption was attenuated to a degree directly related to length of neuroleptic pretreatment, consistent with long-term antipsychotic efficacy of neuroleptics. Pretreatment with dopamine antagonists and agonists suggests that the mechanism involved in DMT blockade may be through denervation supersensitivity of dopamine receptors. In the case of PEA, attenuation of stereotyped behavior paralleled the clinical potency of neuroleptics on a mg/kg basis and in terms of relative ratios. Complete tolerance did not develop to DMT disruption or PEA stereotypy, consistent with the chronic course of schizophrenia. However, rapid tolerance develops to PEA disruption of shuttlebox if doses subthreshold on PEA induced stereotypy. The drug induced behavioral models reported here are of particular interest because they involve endogenous compounds implicated in the pathogenesis of schizophrenia and there is some degree of pharmacological isomorphism between these models and the clinical condition of schizophrenia.

B. Clinical

The use of GLC-MS in psychiatric disorders is particularly important as endogenous compounds of interest are frequently present in low concentrations and specificity is of considerable concern. This is particularly true for dimethyl tryptamine (DMT) and its potential role in schizophrenia. The indoleamine DMT is a known psychotomimetic agent, and has been reported to be present in human blood and urine. Moreover, an enzyme system capable of forming DMT has been demonstrated to be present in man.

Using this assay, we found no differences in DMT plasma concentrations between groups of normals, chronic schizophrenics, acute schizophrenics, and psychotic depressives. In fact, we were unable to detect DMT in plasma at all except in one normal and one psychotic depressive. Kaplan et al. observed that DMT blood concentrations following administration of a psychoactive DMT dose (0.7 mg/kg) to human subjects were low, peaking at less than 100 ng/ml at ten minutes post-dose (the time of the greatest subjective response) and dropping off rapidly thereafter.

One possible explanation for the failure to find a correlation between blood concentrations of DMT and schizophrenia may be that endogenous DMT concentrations are exceedingly small. Any differences in DMT concentrations between normals and schizophrenics would be missed if the higher level were still below the detection limit of the assay employed. Several refinements in the original assay procedure improved the detection limit to 0.05 ng/ml whole blood. In this study 50% of the patients and 40% of the controls gave measurable DMT concentrations. In the work described here, the assay procedure has been modified extensively, resulting in a practical sensitivity limit for DMT of 10 pg/ml whole blood.

It has been hypothesized that phenylethylamine (PEA), a naturally-occurring amine, may be of etiologic importance in some cases of schizophrenia. PEA is structurally and pharmacologically similar to amphetamine. Furthermore, normal persons who ingest amphetamines in sufficient doses may develop a symptomatology that is clinically indistinguishable from paranoid schizophrenia.

In animals PEA produces stereotypies very similar to those produced by amphetamine. Both amphetamine- and PEA-induced stereotypies in animals are blocked by neuroleptics that are also clinically useful in treating schizophrenia. The stereotypies are blocked by doses approximately equal to the neuroleptics' relative potencies in schizophrenia.

To investigate the relationship between PEA and schizophrenia we collected 24-hour urine samples from 23 chronic schizophrenics and 23 normals. All patients received a RDC diagnosis of chronic schizophrenia and had been chronically hospitalized for at least one year without return to premorbid level of functioning. Eleven patients were subgrouped as paranoid schizophrenic and 12 as nonparanoid schizophrenic as determined by historical review, semistructured interview, and staff consensus diagnoses based on DSM II categories. While many patients were on phenothiazines, we found that phenothiazines did not consistently alter urinary PEA levels in eight patients studied on and off medication. The one-way analysis of variance test was significant for 24-hour PEA urinary excretion for paranoid, nonparanoid, and normal subjects (F=6.21, p=.0043, with Brown-Forsythe Unequal Variance correction F=3.98, p=.04). The 11 paranoid chronic schizophrenics' mean ± SEM PEA excretion of 18.1±5.4 ng/24 hrs was significantly higher than the 23 normal controls' excretion of 5.6±.5 ng (two-tailed t-test for unequal variances p=.044). The nonparanoid subgroup (8.6±2.4) was not significantly higher than the control group.

Since MAO is decreased in the platelets and lymphocytes of some chronic schizophrenics, particularly paranoid schizophrenics, it is possible that such a reduction might represent a decreased capacity to metabolize PEA and thereby lead to PEA's accumulation. The finding of increased PEA in urine of chronic schizophrenics, offers some indication that PEA may be an endogenous amphetamine.

Phenylacetic acid. A rapid, sensitive method for the quantification of free and total PAA in 10 μl of urine by mass fragmentography was developed. The method is very reproducable showing an interclass correlation of 0.97 seg at >.001 on nine samples done three times. Total PAA was determined in 12 and 24-hour collections stored at -40°C. All determinations were done in duplicate (410 determinations, interclass correlation .92-.97 on duplicates).

The schizophrenic patients had a significantly lower (+=2.05, $p<.05$) urinary PAA than normals (schizophrenics 95.5±15.5 mg, N=24, and normals 146.2±19.7, N=22). Eight patients consistently showed extremely low PAA excretion, and only three normals showed low excretion.

Using the matched samples test, no significant difference in PAA was found between the 12-hour day urine collections (9:00 a.m. - 9:00 p.m.) and the 12-hour night collections in normals (t=1.0). However, the day urine collections in 12 patients were significantly lower in PAA than the night collections (t=2.44, $p<.05$). No significant difference was found in urine PAA before and after ingestion of 10 ounces of chocolate (0.19 mg PEA) in five normals but headaches were precipitated in three.

To further explore our interest in phenylethylamine metabolism and psychiatric disorders, urines were collected from 9:00 a.m. to 9:00 p.m. on one day and 9:00 p.m. to 9:00 a.m. the following day from 13 normal adult volunteers working at the National Institute of Mental Health. After collection, the urines were mixed with 10 ml 10% EDTA, the volume measured and aliquots frozen at -40°C until analyzed. These urines were analyzed for total PAA. Twenty-four hour urines were also collected from 24 normal subjects and analyzed for free, total and conjugated PAA. The addition of 10% EDTA did not alter the urine pH. All urines were individually checked and found to have pH values between 5 and 7 with a mean of around 6. Storage of urine at -40°C did not appear to effect free PAA nor PAG concentrations. Analysis of some urines repeated about 6 months apart gave essentially the same results.

To test the reliability of the assay, samples from ten individuals were aliquoted into two sets of vials and blindly analyzed one week apart. The intraclass correlations for total, conjugated and free PAA were 0.98, 0.85 and 0.95 respectively. On the other hand, duplicates analysis of several urines carried out in the same batch showed correlation coefficients well over 0.95. The method described here is currently being used to evaluate PAA excretion in mental illnesses and is linear for all values so far examined. We have recently completed the analysis of urines obtained from patients on a variety of antipsychotic drugs and failed to observe a single sample with peaks that emerge near or close to those corresponding to PAA and PAA-d_7.

C. Animal

Six individually caged juvenile Macaca mulatta (Rhesus monkeys) were studied during two sessions before and four sessions after IM injections of PEA. During each session, two observers who were blind to treatment conditions, counted checking (a stereotyped form of hypervigilance manifested by abrupt changes in visual field) and 27 other discrete categories of behavior. Differences between pre-drug and post-drug behavioral counts were computed for each animal.

Single injections of PEA produced dose-related (25-75 mg/kg) significant increases in checking and lip smacking (another stereotyped behavior) as well as decreases in goal-directed behaviors such as grooming. These effects, which are often accompanied by variable increases in other stereotyped behavior such as rocking, chewing, gnashing and repetitive pacing were maximal at 15 minutes post injection, still substantial at 30 minutes, and mostly dissipated by one hour. These effects are similar to those induced by the chronic administration of amphetamines. Of interest is that unlike amphetamines, consistent tolerance did not invariably develop to the repetitive administration of PEA (75 mg/kg t.i.d.) for four or nine days.

Pretreatment with alpha-methyl-para-tyrosine (AMPT) (125 mg/kg), but not with phenobarbital (25 mg/kg) substantially reduced the characteristic effects of PEA. However, when the same AMPT pretreatment was followed by a subthreshold dose of L-DOPA (5 mg/kg) typical PEA effects were restored. This finding suggests that at least some PEA effects require intact dopaminergic mechanisms and that AMPT does not block PEA receptors.

In a present study, we demonstrated that pretreatment with either a Type A MAOI, clorgyline, or a Type B MAOI, pargyline, markedly reduced the dose of PEA required to produce checking and a variety of other behavioral changes. The present results indicate that pretreatment with MAO inhibitors, clorgyline and pargyline, potentiate the effects of PEA on Checking. That is, doses of PEA (5 or 10 mg/kg), which we previously showed to be without effect, now produced significant Checking values in pretreated animals. Neither inhibitor by itself produced Checking.

Although PEA is a better substrate for MAO Type B than for Type A, both clorgyline (a Type A inhibitor) and pargyline (a Type B inhibitor) appeared to potentiate the behavioral effects of exogenously administered PEA on Checking. The reasons for these observations are not fully known at this time. Previous studies have suggested that PEA releases dopamine: i.e., the behavioral effects of PEA were blocked not only by pretreatment with alpha-methyl-para-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase but by pretreatment with dopamine receptor blockers such as chlorpromazine, pimozide and haloperidol. Since both Type A and Type B MAO inhibitors may inhibit the metabolism of dopamine, the present results are compatable with the hypothesis that PEA's effects are mediated by dopamine. Further studies would be required to substantiate this hypothesis.

D. Convulsive Properties

Methionine has been found to alter the behavior of animals and to adversely affect the behavior of schizophrenic humans. It has been suggested that these effects are due either to (1) increased availability of methyl groups for the production of methylated amines; or (2) to the accumulation of toxic metabolites of methionine, such as homocysteine and homocysteic acid, which have convulsive properties. If this latter hypothesis is correct, methionine itself would be expected to have the ability to promote convulsions, either alone or when administered to animals that have been "primed" with subconvulsive dosages of other known convulsants.

Initially, the inability of methionine alone to induce convulsions was demonstrated by (1) administrating solutions containing 250, 500, or 1000 mg/kg of methionine (i.p. or s.q., N=243) and observing the animals for four hours; (2) administering suspensions of 1,2, or 4 mg/kg of methionine i.p. (N=8 per treatment), and observing the animals for four hours; and (3) infusing methionine (50 mg/ml) into mice via the tail vein at varying rates in total dosages of up to 10 gm/kg (N=8). In no case was a convulsion, tremor, or any convulsive-like phenomenon observed in any mouse given methionine alone. Alterations in behavioral activity were observed; the 250 mg/kg dosage caused small increases in behavioral activity, while the larger dosages (500-4000 mg/kg) caused decreases in activity. However, when methionine (1000 mg/kg) was given to animals pretreated with subconvulsive or threshold convulsive doses of homocysteine or pentylenetrazol, a pronounced enhancement of convulsive responses was observed: homocysteine seemed to be more effective than pentylenetetrazol in this respect. The convulsion-promoting effect of methionine was not shared by other amino acids, including glutamate, glycine, cysteine, tryptophan, and others. On the other hand, ethionine, an analogue of methionine, was found to enhance convulsions about as effectively as methionine. These results show that methionine has convulsion-promoting properties under certain conditions, and support the suggest that the organicity produced by methionine clinically is due to the accumulation of metabolites such as homocysteine.

Anticonvulsant Properties of Betaine. Betaine has been found to block the induction of convulsions produced by homocysteine; the reason for this effect has been presumed to be that betaine acts as a source of methyl groups for the methylation of homocysteine. This hypothesis would not predict that betaine would have general anticonvulsant properties. A series of experiments was performed to test this possibility. It was found that betaine (380-1540 mg/kg) partially blocked not only those convulsions induced by homocysteine, but also the convulsions induced by electroconvulsive shock and pentylenetetrazol. If the reason for the anticonvulsant activity of betaine is indeed that it reduces the ratio of free homocysteine to free methionine in the body, the present finding suggest that homocysteine plays a role in the regulation of neuronal excitability under "normal" circumstances; e.g., in the absence of an administration of exogenous homocysteine.

XVI. Low Platelet Monoamine Oxidase Activity and Schizophrenia

The finding that blood platelet monoamine oxidase (MAO) activity is low in some schizophrenic patients has led us and others to inquire as to its significance. As part of this endeavor, there has been a continuous effort to determine if this low enzyme activity was due to one of the large number of artifacts that have plaqued the investigation of biological factors related to psychiatric disorders. The enzyme has been assayed in the laboratory of Dr. Dennis Murphy, who, in addition to his interest in the enzyme in schizophrenia, has been using the platelet as a model for synaptosomes.

A. MAO Assay in Blood Platelets

In our initial studies, venous blood was collected in heparin, or more recently, in Becton-Dickinson Company Vacutainer tubes containing ACD (acid-citrate-dextrose), NIH Formula A, and platelets separated and stored according to previously described methods. MAO activity was determined using tryptamine-2-^{14}C HCl ($8x10^{-5}$M, 8.9 mCi/mM) as substrate and was expressed as nM of tryptamine coverted per mg of platelet protein. The product was indoleacetaldehyde, whose formation was linear with time and enzyme concentration.

During the last several years, our samples were also obtained in the ACD solution, but the MAO activity was determined in platelet rich plasma. The present assays uses benzylamine-^{14}C ($4x10^{-4}$ mCi/mM) as substrate, and is expressed as nM of product per 10^{-8} platelets per hour. Benzylamine is a good substrate for both platelet MAO and an entirely different enzyme occurring in the plasma. Because of this, platelet rich plasma samples with and without the MAO inhibitor pargyline are run. Pargyline ($2.4x10^{-4}$M) completely inhibits the platelet enzyme without affecting the plasma enzyme. The value of sample with the inhibitor is substracted from the sample without--the difference being the platelet activity.

The advantage of the platelet rich plasma assay over the previous one is in not having to determine protein which can easily be contaminated with red and white cells.

B. Stability of Enzyme

Because at times it is not possible to process blood samples or run enzyme assays immediately, it is necessary to know the stability of the enzyme. Comparisons of blood samples processed immediately or kept on ice or at room temperature for five hours revealed no change in activity. Similarly, once the platelet rich plasma is separated, MAO activity remains stable for at least 30 days when kept at -70^{0}C.

C. Comparison of Assays

Pearson correlations were run for 48 normals and 20 chronic schizophrenics between the two assays. The correlation for the normals was .85 while that for the chronic schizophrenic patients was .76. For the combined group the correlation was .83. All our platelet MAO activities are expressed in benzylamine values with a correction (derived from the normals) for values determined using the tryptamine assay.

D. Subjects

Normal bloods were drawn from normal volunteers and Hospital personnel at both the Clinical Center in Bethesda, Maryland and the William A. White Building of Saint Elizabeths Hospital. The bloods from the acute schizophrenics largely came from a research ward at the Clinical Center while those from the chronic schizophrenic patients came from special research wards at the William A. White Building.

E. Age and Sex

Others have reported that females had 10 percent higher platelet MAO activity than males. Our sample females have a slight but consistently higher MAO activity although this difference is statistically significant only for the young adult group.

F. Drugs

Although early experiments with chlorpromazine in intact systems indicated that chlorpromazine had some effects on amine degradation, this seemed to be due to poor amine accessibility to the enzyme because of alterations in the cell membrane. Edwards and Burns (1974) found that the I50 for amitriptyline was 2×10^{-4}M for human platelet MAO *in vitro* when benzylamine was used as a substrate. The inhibition was competitive.

In studies on patients given neuroleptics, there does not appear to be a consistent difference between when they are on neuroleptics or off, or between patients on neuroleptics and their siblings not receiving drugs.

G. Hormones

One study using only two points found no alteration in platelet MAO activity during the human menstrual cycle. In another study which utilized samples collected three times a week, there were small (23 percent) peak to trough variations in activity in the majority of women. The peak MAO activity occurred during the preovulatory interval and the nadir occurred five to 11 days later. A small number of patients taking prednisone have been seen and have normal MAO activities. Similarly, there does not appear to be any correspondence between plasma thyroxin and triiodothyronine concentrations in psychiatric patients with low and normal MAO activities.

H. Other Factors

The hematocrit, hemoglobin and platelet counts, serum iron, and iron binding capacity were not systematically different in patients with low and normal MAO activity. Other platelet enzyme activities including succinate dehydrogenase, lactate dehydrogenase and cytochrome C reductase were no different in chronic schizophrenic patients with low MAO than they were in controls.

I. Chronic Schizophrenics

To date we have studied platelet MAO activity in 104 male chronic schizophrenic patients. Their mean ±S.E. MAO activity was 7.86±.56 while the value for 348 normal males (ages 18-50) was 11.04±.29. This difference using a two-tailed "t" test, was significant at $p<.001$. The mean for the 15 female chronic schizophrenics was 7.04±1.06 while that for 332 normal females was 13.29±0.29 ($p<.001$).

The effect of chronic hospitalization on platelet MAO was studied at the Palo Alto VA Hospital where 50 normal controls were compared to 16 patients hospitalized for traumatic accidents to their spines. There was no difference between MAO activity between groups but 39 chronic schizophrenics had decreased platelet MAO activity.

At least 26 studies have been published concerning the enzyme, monoamine oxidase (MAO), in platelets and white cells of chronic schizophrenic patients. Although at times the deviation from controls has been very small, in all but 4 instances a decrease in MAO activity has been found. In 19 studies, the reported decrease was statistically significant. Using the sign test, the chance of 22 of 26 studies demonstrating a decrease is less than 1 in 4,000 and the possibility of 19 of 26 being statistically significant at the 0.05 level by chance alone is less than 1 in 10 (binomial test). In spite of this evidence, the finding continues to be thought of as controversial. We believe that what is controversial about the finding is not whether or not platelet MAO activity is decreased in some schizophrenics, but 1) to what degree, 2) in which patients, and 3) what, if any, biological significance this decrease might have.

J. Acute vs. Chronic Schizophrenics

To determine whether acute schizophrenics have a reduction in platelet MAO as did the chronic schizophrenics, platelet MAO of 27 acute schizophrenics were studied. The 10 male acute schizophrenics (ages 16-30) had a mean platelet MAO activity of 10.68±78 which was no different than that of normal males. The 17 female acute patients had a mean of 13.85±1.12 which was no different from that for the normal females.

K. Subgroups of Chronic Schizophrenics

Platelet MAO in two groups of chronic schizophrenic patients (N=35, x=7.52; N=42, x=7.86) was significantly lower than in controls (N=580, x=12.14). Values for those patients diagnosed as paranoid, (N=8, x=4.47) were significantly lower when compared to non-paranoid schizophrenics (N=27, x=8.43) in a retrospective study. These findings were confirmed in a separate prospective study of 42 chronic schizophrenics (paranoid and paranoid features N=24, x=6.40 compared to non-paranoid N=18, x=9.81). Schildkaut found low platelet monoamine oxidase to be associated with auditory hallucinations and delusions, usually of a persecutory quality. Twelve out of 16 of these low monoamine oxidase patients were designated paranoid. Reduced platelet MAO activity in normal subjects appears to be associated with a greater tendency towards psychopathology and a perceptual behavior study which is similar to that of paranoid schizophrenics. These data could indicate that paranoid schizophrenia may represent a separate disorder from the other schizophrenias. The correlation between paranoid schizophrenia and low platelet MAO activity suggests some biochemical difference between the different subgroups of schizophrenia.

L. Specificity with Regard to Other Illness

Since the various psychiatric diagnostic classifications are thought to represent different entities, a defect in one should not be seen in the other. The affective illnesses are the group we most diligently contrasted with the schizophrenias.

Sixteen male and 13 female unipolar depressed patients were found to have platelet MAO activity that was no different from our controls while 16 male and 33 female bipolar I depressed patients had reduced activity ($p< .001$). The bipolar patients had a negative skew which was not as large as that of the chronic schizophrenics. Using six units of MAO for the male normals and eight units for the normal females as a point of cut-off, a point under which less than 10 percent of normals fit, 39 percent of male chronic schizophrenics and 60 percent of the female chronic schizophrenics are found (chi square $p<.001$). The bipolar patients also differed from normals ($p<.001$ males; $p<.02$ females). The male bipolar patients were not significantly different from the male chronic schizophrenic patients, but the percentage of female chronic schizophrenics with MAO activity below eight units was greater than the female bipolar I patients. Thus, although bipolar I patients using the arbitrary cut-off have a mean MAO which is low, it is not clear whether they represent a separate group from the chronic schizophrenic patients.

M. Twins Discordant for Schizophrenia

To exclude the possibility that the low platelet MAO activity seen in the chronic schizophrenics was caused by nongenetic factors as opposed to genetic ones, we studied monozygotic twins discordant for schizophrenia. If the low platelet MAO activity is due to some aspect of being schizophrenic, low platelet MAO should only be present in the schizophrenic twin. Thirteen schizophrenic index twins, all of whom had been hospitalized at least once for schizophrenia and had been extensively studied by Dr. William Pollin and his associates, were examined along with their non schizophrenic co-twins. At the time of the study, one patient resided within a hospital while five were in remission. Four of the patients had had acute forms of schizophrenia and six patients were not receiving any antipsychotic medication. The nonschizophrenic co-twins had never been hospitalized for a behavioral disorder and were functioning well within their families and communities, except for one individual with borderline psychosocial adjustment.

Only two twin pairs were living in the same household and nine of the co-twins were living in different cities. It was, therefore, necessary to obtain and prepare the blood samples at various facilities throughout the country. Because of this, normal control samples were obtained at the same time as those from the twins. They were coded and shipped to the Laboratory. All samples were batched together before assay. (In all studies samples are run in a manner so that their origins are unknown to all investigators until the final calculations are made.)

The MAO activity of the 23 normal controls was the same as that obtained at that time for previous controls (16.02). The schizophrenic twins (9.88, $p<.005$) and nonschizophrenic twins (12.08, $p<.05$) were significantly lower than the normals, but there was no difference between the twin groups. There was a significant Pearson correlation ($r=.67$, $p<.01$) between the MAO activities in the schizophrenic and nonschizophrenic twins.

There were four pairs of twins whose MAO activities were especially low. The indexes of all four of these twin pairs were chronic schizophrenics while the more acute patients had MAO activities closer to normal. The severity of impairment (based upon number and duration of hospitalizations) was rated on a 5-point scale for the schizophrenic twins by an investigator with no knowledge of the platelet assay. A forced rank order was then made between the numerical ratings, with the highest number given to the most ill patients. Using this order, and comparing it to the MAO activities, a Spearman rank order correlation of -.54 ($p<.05$) was found.

Platelet MAO activity was also examined in nine monoxygotic and 10 dizygotic normal twins. The intraclass correlation coefficient was 0.88 for the monozygotic and 0.45 for the dizygotic twins. The correlations were significantly different from one another (Mann Whitney U $p<.001$). Same-sex siblings matched for age and sex were about the same as the dizygotic twins while unrelated similar to those reported by Nies, Robinson, Lamborn and Lampert (1973) for normal monozygotic and dizygotic twins using benzylamine instead of tryptamine as a substrate.

Taken together this data indicates that platelet MAO activity is in a large part determined by genetic factors and that the low platelet activity seen in the chronic schizophrenics is not secondary to being ill, but is genetically related to the liability to be schizophrenic.

N. Family Studies

In order to further study the relationship of low platelet MAO to schizophrenia the blood of first-degree nonschizophrenic relatives of index schizophrenics (N=16) was collected. Dividing schizophrenic indexes at their median, the low indexes had a mean of 4.64 and the high indexes had a mean of 10.81. The mean MAO activity for the low index first-degree relative was 8.56. The mean activity for high index first-degree relatives was 11.84. The difference between the means for the low vs. high first-degree relatives was significant ($p<.02$; Mann Whitney U). No attempt was made in this small sample to account for sex differences. These differences between nonschizophrenic first-degree relatives of high and low schizophrenics is another indication of the genetic nature of platelet MAO activity.

Ongoing family studies for the past two years presently include studying MAO in platelets and lymphocytes in first-degree relatives of schizophrenics. At present, findings are inconclusive. As well, a collaborative effort is being established with other Divisions of the Hospital to study the inheritance of schizophrenia by hospitalizing first-degree relatives with schizophrenia.

O. Physical-Chemical Studies

In one study, we determined the rate of heat inactivation of the MAO in a group of chronic schizophrenic patients (N=22) and normal subjects (N=13). Aliquots of platelet MAO were subjected to different temperatures between 56 and 62 C for five minutes. Analysis of the data revealed no significant differences in the rate of heat inactivation between the normal and schizophrenic groups confirming an earlier study from this laboratory. However, when the schizophrenic group was divided into paranoid schizophrenic and chronic undifferentiated schizophrenic (CUS) groups, differences became apparent. The MAO in the paranoid group was most resistant to heat while the MAO in the CUS group was the least resistant; the MAO in the normal group was intermediate in its rate of heat inactivation. There was a significant difference ($p<.05$) between the paranoid and nonparanoid (CUS) groups. Neither schizophrenic group differed significantly from the normal group. However, the combination of the normal plus the CUS group different significantly from the paranoid group ($p<.035$).

In a second study, we have attempted to replicate a finding reported by Berrettini and Vogel that an inhibitor of platelet MAO is present in the plasma of schizophrenic patients that have low MAO activity. If their report were correct, it would provide a mechanism that would explain the reduced MAO activity found in schizophrenic patients. However, we have not been able to replicate their results. In fact, in five of six experiments we have found a slight increase in MAO activity when normal platelet MAO is preincubated with plasma from schizophrenic patients (effect of chronic plasma on Km and Vmax, 1.5 ± 0.05 and 22.1 ± 2.46; effect of schizophrenic plasma on Km and Vmax, 1.8 ± 0.28 and 24.9 ± 1.57). We would conclude from our own studies that an inhibitor does not appear to be present in schizophrenic plasma.

In a third study, we are attempting to replicate the finding of a reduced Km in the platelet MAO in a group of paranoid patients compared to a group of normal subjects. Although our preliminary findings are in the right direction, certain methodological problems have become apparent and need to be resolved before we can complete the study.

If this finding can be firmly established in the schizophrenic group, we then plan to proceed and to measure the Km in a group of alcoholic patients and in a group of bipolar depressive patients. The overall purpose would be to determine if the decrease of Km in the schizophrenic patients observed is unique to that patient group or is shared by the other two patient groups that have also been reported to have a reduced platelet MAO activity.

Heat Inactivation: Most studies have reported that chronic schizophrenic patients have reduced platelet monoamine oxidase (MAO) activity. Possible environmental and genetic factors that might cause the lower activity have been the subject of intense investigation. On the one hand, most environmental factors, such as drugs and hospitalization that might cause the decreased MAO activity have been excluded. On the other hand, it appears that the level of MAO activity in both normal and schizophrenics is, at least in part, due to genetic factors. Given the possibility of a genetic contribution, it seemed reasonable to determine if the MAO itself was altered. To determine this, we have examined some physical-chemical properties of MAO. We and others have shown that with some substrates the Km is altered in the MAO of chronic schizophrenic patients. In the present study, we have attempted to examined the rate of heat inactivation of platelet MAO in a group of normal subjects and chronic schizophrenic patients.

Berrettini and Vogel describe evidence for an inhibitor of platelet monoamine oxidase (MAO) in the blood of chronic schizophrenic patients. They suggest that this endogenous inhibitor may be responsible for the decreased platelet MAO activity reported in chronic schizophrenics. We, however, failed to demonstrate the presence of this inhibitor.

The blood from eight patients with low MAO activity was used. The plasma from two patients was provided by Dr. Berrettini. The other six patients were hospitalized at Saint Elizabeths Hospital where they received a diagnosis of schizophrenia both by Feighner and Research Diagnostic Criteria. The patients who satisfied these criteria had a chronic illness and were unable to function in the community. Additionally, the clinical staff who best knew the six patients rendered a consensus subtype diagnosis of paranoid schizophrenia based on DSM-II categories. Since Berrettini and Vogel did not use drug free patients and since neuroleptic drugs seem to have little effect on MAO activity, the patients used in the present study were not neuroleptic free. Staff and laboratory personnel were used for the control population. The procedure for determining the presence of an inhibitor was that employed by Berrettini and Vogel. They indicate that the inhibitor is present in the platelet-poor plasma (PPP) of schizophrenic patients who have low platelet MAO activity. To briefly describe their procedure, three aliquots of platelet-rich plasma (PRP) from a normal were used in each experiment. One aliquot of the normal PRP was unmixed. The second PRP aliquot was mixed with PPP that was obtained from a second normal control and which presumably contained no inhibitor. The third PRP aliquot was mixed with the schizophrenic PPP. All three samples were incubated for 10 min. at 37 . The samples were centrifuged to obtain the platelet pellet which was then washed and resuspended in buffer. The kinetic parameters (Km and Vmax) for the three samples were determined.

The enzymatic assay method of Wurtman and Axelrod was used. Radioactive tyramine (tyramine [1-^{14}C]) and radioactive dopamine (dihydroxyphenylethylamine hydrobromide, 3,4 [ethyl-^{14}C]) were obtained from New England Nuclear.

When tyramine was employed as a substrate, five concentrations were used that range from $10X10^{-4}$ to $1X10^{-4}$M. The reaction was stopped with perchloric acid and the product was extracted with toluene. When dopamine was employed as a substrate, five concentrations were used that ranged from $8X10^{-4}$ to $0.5X10^{-4}$ M. The reaction was stopped with hydrochloric acid and the product extracted with toluene: tetra-hydrofuran (2:1, V:V). Protein was determined by the method of Lowry et al.

The Km's and Vmax's were obtained by fitting data with a PDP-11 computer[2]. to a rectangular hyperbola according to the iterative procedures of Bliss and James. This method yields the same results as those procedures developed by Cleland. The data were then further analyzed with a two-tailed paired t-test.

The data indicate that there were no significant differences among any of the groups in either the Km or Vmax measurements. Berrettini and Vogel reported that treatment of normal platelets with schizophrenic platelet-poor plasma reduced both the Vmax and Km of normal MAO. We did not find reductions; if anything, our data indicate that the schizophrenic plasma produced nonsignificant increases in MAO activity. More specifically, the schizophrenic plasma produced increases in the Vmax in 6 out of 8 cases when tyramine was used as a substrate ($p<.06$). In addition, the two schizophrenic plasmas provided by Dr. Berrettini also produced increases in the Vmax.

It is not clear why we were unable to replicate the findings of Berrettini and Vogel. If there is an inhibitor, there must be factors affecting the procedure of which Berrettini and Vogel are not aware. One possible factor involved could be the procedure used in analyzing the data. WE used the objective mathematical method of Bliss and James. Berrettini and Vogel used Linewearver-Burk plots in which the straight line was fitted by eye to obtain their Vmax and Km values. This plotting procedure has been shown to be subject to substantial error. Dowd and Riggs conclude that, "The marked inferiority of the Linewearver-Burk plot strongly suggests that it should be abandoned as a method for estimating Km and Vmax from unweighted points, whether the points are fitted by eye or by the method of least squares." Furthermore, if Berrettini and Vogel did not plot their data as a blind procedure, an additional error could have been introduced.

P. Relationship of Platelet MAO in Brain MAO

The number of types of MAO present in the brain is an unresolved question. Original studies indicated that there might be as many as four isoenzymes of MAO within the brain; however, Houslay and Tipton (1973) suggested that the multiple enzymes found using polyacrylamide-gel electrophoresis might be produced artifactually in a preparatory step. The authors state that these multiple MAO forms may represent a single protein with varying amounts of phospholipid attached; the amount of phospholipid may, in turn, determine the enzyme mobility during electrophoresis.

Another approach to this problem has come from the finding that selective inhibition of MAO can be used to identify multiple forms of MAO. Clorgyline was the first MAO inhibitor employed in this manner. It was found that tissue homogenates pretreated with increasing concentrations of clorgyline produced stepwise inhibition to the substrate tyramine, suggesting the presence of an enzyme sensitive to the inhibitor and one relatively resistent to it. Considerable more work has been done to identify these enzymes and for convenience they are called Type A and Type B.

The preferred substrates for the Type A MAO are norepinephrine, serotonin, and normetanephrine, while for the Type B they are benzylamine and β-phenylethylamine. Dopamine, tyramine and tryptamine are common substrates. Clorgyline, Lilly 51641, and harmaline are specific inhibitors of Type A while deprenyl and pargyline inhibit Type B. Most of the other MAO inhibitors used in clinical medicine, however, are nonspecific. Platelet MAO, forming one band on electrophoresis, appears to be a single enzyme with Type B characteristics. That is, the platelet enzyme demonstrates only a single sigmoid curve for inhibition using deprenyl, pargyline, or clorgyline. Furthermore, the platelet enzyme is 200-fold more sensitive to inhibition by deprenyl than clorgyline. The conclusion that the platelet enzyme is a simple Type B MAO is also supported by the high activity of the platelet enzyme with the relatively specific MAO-B substrates, benzylamine and β-phenylethylamine, in contrast to its lesser activity with serotonin, an A substrate.

Q. Function

To determine whether or not the low platelet MAO activity might have a functional significance, platelet-rich plasma was obtained from 20 normal controls (age 29.6±1.1) and 16 chronic schizophrenic patients (27±1.4). Since plasma serotonin is almost entirely contained in the platelet the values were expressed in platelet units (ng/108 platelets). The platelet counts for the normals and schizophrenics were the same. When the patients had been off phenothiazines (N=12) for 30 days or longer the serotonin concentration was higher (80±4), while when the patients were taking phenothiazines (N=12) their platelet serotonin concentration was normal (90±9). The reason for the discrepancy between patients taking and not taking phenothiazines may be due to the latter's ability to block serotonin uptake into platelets. Nevertheless, since serotonin is not a good substrate for platelet MAO (Km 10^{-3}) other substrates might be much more affected.

Increases in plasma prolactin concentrations produced by alpha-methyl-para-tyrosine, a catecholamine synthesis inhibitor, varied inversely with baseline platelet monoamine oxidase activity in 12 chronic schizophrenic patients. Two subsequent studies with 39 normal volunteers and 19 unmedicated chronic schizophrenic patients were conducted to test for a relationship between platelet monoamine oxidase activity and plasma prolactin concentration. Plasma prolactin concentrations were found to vary directly with platelet monoamine oxidase activity in normals with low monoamine oxidase activity and patients with chronic schizophrenia. No relationship was found in normals with high platelet monoamine oxidase activity. These data suggest that platelet monoamine oxidase activity reflects monoaminergic activity in the tuberoinfundibular system which in turn affects plasma prolactin concentrations. This relationship may be particularly important in patients with low platelet monoamine oxidase activity such as some chronic schizophrenics.

R. Subclass of Schizophrenia

In 1974-1975 we undertook a restrospective study of 43 patients we had examined to look at who the patients were with low platelet MAO. Information was obtained regarding circumstances and age at onset of illness, presenting problems, diagnoses and family history of mental illness. The patient's medical and surgical history was reviewed, as well as laboratory data which included EEG's and skull x-rays. Mental status examination on admission and throughout hospitalization was summarized, as well as changes observed on medication. This information was gathered and coded without knowledge of platelet MAO activity.

Two types of statistical analyses were employed. The first approach correlated MAO activity with a variety of clinical variables using the Pearson product-moment correlation coefficient. The second approach looked for differences between groups of patients with low and high MAO activity. In the second approach, the MAO value of 9.16 units was arbitrarily chosen to divide the chronic schizophrenic patients into high and low MAO groups (12 patients in the high group; 31 patients in the low group). 9.16 is two standard errors above the female chronic schizophrenic mean (8.04 and slightly higher than two standard errors above the male schizophrenic mean (7.86). The term "high MAO" in this study refers to high MAO activity for chronic schizophrenic patients. The high MAO group has a mean ± SEM of 13.54±1.06, which is comparable to values for the normal population. The low MAO group had a mean of 5.47±.37. All patients were diagnosed chronic schizophrenics with a mean hospital stay of 9.3 years, although length of hospitalization within the sample did not correlate with MAO activity ($r=.11$). MAO activity did not correlate with age at onset of symptoms ($r=.06$), age at first hospitalization ($r=.12$), length of hospitalization ($r=.11$), or with problems at work ($r=.20$), school ($r=.07$), or marriage ($r=.12$).

Schizophrenic patients with low MAO had a history of more seizures ($r=.31$, $p<.05$) than patients with high MAO. Of the 41 patients from whom we had an adequate history of seizures, 8 of 30 low MAO schizophrenics had at least one seizure during hospitalization, compared to 1 of 11 high MAO schizophrenics. The high-low difference was not significant using the Fisher Exact Probability Test. None of the patients was on anticonvulsive medication. The possibility of abnormal brain dysfunction correlating with low MAO was reflected in the significant negative correlation between MAO and the number of abnormal EEG's ($r=.32$, $p<.05$). Of those 36 patients who had EEG records available, 10 of the 25 low MAO schizophrenics had at least one abnormal EEG, compared to none of the 11 high MAO schizophrenics (Fisher Exact Probability Test, $p<.01$). The data indicate that low MAO activity in chronic schizophrenics is associated with an increase in abnormal EEG's and perhaps an increase in seizures. This abnormal brain activity is, however, not associated with other medical or asurgical problems, or with any abnormalities in routine blood studies.

In regard to the mental status examination, the patients with low MAO activity were reported to have more ambivalence ($r=.32$, $p<.05$) but not any difference in severity or frequency of thought disorder ($r=.02$), associational defects ($r=.10$), autistic behavior ($r=.03$), hallucinations ($r=.09$), instances of self-mutilation ($r=.04$) or suicide attempts ($r=.24$). No evidence for differences in treatment response related to MAO activity was found ($r=.05$). Mental illness in relatives did not correlate ($r=.19$) with platelet MAO activity and was not different between the high and low MAO groups. However, it might be impossible to determine such a correlation from the relatively small sample. This is compounded by the inherent difficulties in determining a complete accounting of family incidence of mental illness from chart analysis.

All of the paranoid schizophrenics had low MAO. None of the high MAO activity schizophrenics received a DMS II diagnosis of paranoid schizophrenia during their hospital stay; 7 of the 30 low MAO group did (Fisher Exact Probability Test $p<.05$.) This difference was further substantiated when the means of MAO activity were compared between those who received a diagnosis of chronic paranoid schizophrenia and those who did not. The paranoid schizophrenics have a mean MAO activity of $4.8\pm.46$, which is significantly lower than the nonparanoid schizophrenic values of $8.6\pm.75$ (t-test, $p<.03$). This difference was also reflected in symptoms characteristic of chronic paranoid schizophrenia, such as paranoid delusions and increased hostility, but not in other less specific symptoms.

From this study emerged a picture of a low platelet MAO activity schizophrenic who is more frequently diagnosed as paranoid, as having more paranoid type delusions, and as having abnormal brain activity reflected by increased incidence of seizures and abnormal EEG's.

In order to further examine these findings, 42 new patients were selected on the basis of definite diagnosis of schizophrenia who had active symptoms but without regard to subtype. Patients with a diagnosis of organic brain syndrome or mental retardation or other cerebral pathology, including severe alcoholism, were excluded in order to focus on the paranoid dimensions. The 42 subjects were drawn from a sample of 65. Fifteen were excluded because of their history, eight others met criteria but refused to take part in the study. Seven of these eight refused because of apparent suspiciousness of distrust of the experimental procedure.

All patients met Research Diagnostic Criteria and 41 of 42 met Feighner's criteria for a diagnosis of schizophrenia. The mean age was 35, mean length of hospitalization was 401 months, and all patients had, at one time, received phenothiazine treatment. All patients had a chronic course and 40 of the 42 had a gradual or insidious onset of psychosis. Twenty-nine were males and 13 females. MAO values were 6.7 and 10.6 units for males and females, respectively, compared to mean MAO's of 11.04 and 13.29 for normal males and females.

The difference between the schizophrenics and normals was significant at p< .01. The clinical staff who best knew the patient rendered a consensus diagnosis based on DSM II categories. A secondary diagnosis was reached for cases of chronic, undifferentiated schizophrenia. All patients underwent a semi-structured interview by Rosenthal. Interviews were conducted at a time as close as possible to blood drawings, and for most patients were done on one occasions over a 60-minute period. Repeated interviews were occasionally required to obtain the necessary information.

The information was gathered and coded without knowledge of MAO values. The data indicate that patients diagnosed as paranoid schizophrenics had a mean platelet activity MAO level of 5.4 for males and 8.9 for females, compared with 8.6 and 12.1 for nonparanoid male and female schizophrenics. Patients with secondary paranoid features had MAO values of 5.6 for males and 9.1 for females. Values for both paranoid patients and patients with secondary paranoid features were significantly different from those of nonparanoid patients (p<.01).

The variables which best diagnostically divided the paranoid from nonparanoid patients by a linear discriminate function were: degree of suspiciousness, preoccupation with and systematization of delusions, delusions of control, stereotyped behavior, intact memory, rigidity of ideas, and communication characterized by evasiveness and misleading responses. These diagnostically discriminating items are similar to items on the Venables Scale for Paranoid Schizophrenics except for grandiosity which was not a good discriminator in our study.

These prospective findings confirm the retrospective chart analyses. Chronic schizophrenics had lower platelet MAO activity than normals. Paranoid schizophrenics had an even lower MAO activity level, whether the paranoia was determined by clinical diagnosis, by interview, or by a paranoia dimension scale.

S. Discussion

What does it mean to have low platelet MAO activity in view of seemingly normal brain MAO activity? The argument that post-mortem measurement of brain MAO activity does not reflect its true activity because of autolysis has little support since brain MAO seems to be a very stable enzyme. It, of course, remains a possibility that we have either not measured brain MAO activity in the right region or with correct methods, an argument that is not easily dealt with.

Enzyme activity must now be measured in other regions of the body, such as the muscle, lung, liver, and kidney. If these areas turn out to have normal MAO activity while the platelet is low, intriguing explanations will be needed.

Part of the attractiveness of the concept of low MAO activity is that it is congruent with the two most generative biological hypotheses of schizophrenia. The first of these suggests that there is a functional excess of dopamine. A defect of MAO, an enzyme involved in dopamine metabolism, might be able to produce "dopamine excess." The second hypothesis suggests the formation of an hallucinogenic methylated indoleamine (transmethylation). Since the indoleamines as well as their methylated congeners are metabolized by MAO, this theory is also consistent with a finding of low MAO activity. One of many questions that needs to be answered before low MAO activity can be considered causative, is "Why don't the vast majority of people given drugs which inhibit MAO become psychotic?"

XVII. Naloxone

Naloxone was given to 14 schizophrenic patients and five patients with affective disorders were given naloxone (0.4 to 10 milligrams) or placebo intravenously in a double-blind fashion. Physicians' ratings of hallucinations, mannerisms and posturing, conceptual disorganization, psychosis, and mood did not change significantly. A single item, unusual thought content, improved significantly on the naloxone day compared to the placebo day. There was no improvement in mood in affectively ill patients rated either by themselves or by physicians. Naloxone did not markedly improve any patient studied, which suggests that the acute blockade of opiate receptors is not associated with global improvement in psychotic symptomatology.

XVIII. CAT Scan

This study involves quantitative evaluations of computerized tomographic brain scans and quantitative assessments of ventricular volume and cortical sulci size determined from their scans in chronic schizophrenic patients under age 40. This provides a non-invasive, yet accurate means for considering possible abnormalities of these parameters described in prior pneumoencephalographic studies. Preliminary findings suggest that when compared to normal controls, psychiatric patients who do not meet research diagnostic criteria for chronic schizophrenia, and neurological controls, young chronic schizophrenics as a group have larger ventricles and more cortical atrophy. It is also possible that in a small subgroup ventricular enlargement is dramatic.

To investigate the possibility that lateral cerebral ventricular enlargement is associated with chronic schizophrenia, computed tomography head scans from 73 chronic psychiatric patients under age 50 were compared with 56 asymptomatic normal volunteers of similar age. Ventricular size, determined by planimetry, was significantly greater in the subgroup of 58 patients diagnosed with chronic, unremitting schizophrenia than in the control group ($p<.0001$). Forty percent of the chronic schizophrenic patients were outside the control range; 53 percent exceeded two standard deviations of the control mean. Duration of illness and length of hospitalization, variables considered to be proportional to the amount of treatment received, did not correlate with ventricular size. Although patients with a history of ECT treatment had larger ventricles than controls ($p<0.0001$). A group of seven schizophrenic patients who were not chronic also had enlarged ventricles ($p<.006$); 8 patients who were either schizo-affective or not schizophrenic did not differ from controls.

This study demonstrates that lateral cerebral ventricular enlargement is associated with chronic schizophrenia; it suggests that this finding is not a result of treatment. Computed tomography was further employed to investigate whether mild cerebral atrophy involves the cerebral cortex. The widths of the sylvian fissure, the interhemispheric fissure, and three cortical sulci were measured blindly on computed tomography (CT) scans from 75 chronic psychiatric patients and 62 asymptomatic volunteers all under age 50. Using dimensions from the control subjects to define the range of normal size for these structures, 19 (32%) of the 60 patients diagnosed with chronic schizophrenia had at least one abnormality. All 15 patients with other diagnoses were within the control range. Comparing those chronic schizophrenic patients with abnormalities to those without them, there were no significant differences in age, length of illness or treatment, and length of hospitalization. Combining the previously reported data on ventricular size with the present findings, two-thirds of the chronic schizophrenic patients had some abnormality of cerebral structure evident on CT scanning. Ventricular enlargement, however, did not correlate significantly with cortical abnormalities. It is suggested, therefore, that more than one etiology may account for the structural abnormalities found in chronic schizophrenic patients. Furthermore, we also found, totally expectedly, a subgroup of 10 chronic schizophrenic patients with possible pathology of the cerebellum.

The scans of five chronic schizophrenic patients were interpreted by a neuroradiologist as showing definite atrophy of the vermis; in one of these patients, there cerebellar hemispheres were also considered atrophied. Five additional scans, which were interpreted as "questionable", met objective criteria proposed for the diagnosis of vermian atrophy on a CT scan[5]. One of these patients had a history of alcohol abuse. Since cerebellar atrophy is common in chronic alcoholics[6] this patient was excluded from further study. None of the remaining nine patients (four females, five males; mean age 35, range 26-44) had an identifiable cause for this findings; no patient was taking medication other than neuroleptics. Furthermore, careful neurological examination with emphasis on cerebellar function revealed abnormalities in only three patients, two with mild dysdiadokokinesis of the non-dominant hand, and one with a slight positional tremor of both hands.

A growing body of basic science research indicates that the cerebellar vermis is linked anatomically and functionally to areas of the limbic system that have been implicated in schizophrenia. Furthermore, increased dopamine in the limbic forebrain, a neurochemical abnormality consist with the "dopamine hypothesis" of schizophrenia, has been produced in the rat by a lesion of the vermis. It has also been reported that in several schizophrenic patients, electrical stimulation of the vermis had therapeutic effects. Such observations suggest that pathology of the cerebellar vermis may be related to the pathogenesis of some schizophrenic symptomatology.

In a morphometric study of the anterior cerebellar vermis of 46 brains in the Yakovlev Collection, the area of the vermis of five of 12 brains of schizophrenic patients was smaller than any of 11 controls without psychiatric or neurologic disease and eight of nine control with other psychiatric diagnoses ($p<.02$). This finding confirms computerized tomography scan observations in live patients and indicates that some schizophrenics have structural abnormalities of the cerebellar vermis.

Four of 17 chronic schizophrenic for chronic schizoaffective) patients between the ages of 20 and 35, in partial remission, and living in the community were found to have mildly or moderately prominent sulci, as disclosed by computerized tomography (CT scan). These four patients were matched for sex and age with four chronic schizophrenic or schizoaffective) patients without sulcal prominence. The Halstead-Reitan battery (HRB) of neuropsychological tests, including the Wechsler Adult Intelligence Scale (WAIS), was adminstered to these eight patient with the examiner blind to results of the CT scan. Each of the four patients with sulcal prominence considerably exceeded an established cutoff score for brain impairment on the HRB, whereas only one of the control schizophrenics performed in the impaired range, and that just beyond the cutoff. The WAIS Verbal IQ, measured concurrently, was in the normal range for all subjects and similar for the two groups. These findings suggest that mild or moderate sulcal prominence on CT scans among young chronic schizophrenics is associated with impaired neuropsychological functioning.

XIX. Calcitonin and Feeding

Plasma calcitonin (CT) concentrations increase following a meal and following the administration of the gastrointestinal hormones glucagon, gastrin, pancreozymin, and cholecystokinin. A series of experiments were performed to test the hypothesis that this food-induced increase in CT secretion plays a role in reducing subsequent food intake. A single S.C. injection of synthetic salmon calcitonin was found to inhibit the 24-hr intake of rats (up to 58:; $p<0.005$) in a dose-related (12.5-50 U/kg) fashion. This was accompanied by a pronounced diuresis and increase in drinking which were, however, not observed in food-deprived animals. In rhesus monkeys, a single injection of CT (30 U/kg) greatly suppressed feeding for three days and drinking for one day (both $p<0.01$). A retrospective study of humans demonstrated a 2% reduction in body weight ($p=0.03$) during the 12-36-hr following a single S.C. injection of CT (2 U/kg).

To test the hypothesis that inhibition of feeding was due to a direct action on the CNS, we administered calcitonin to rats by intraventricular injection in dosages approximately 25 - 50 fold smaller than those that inhibited feeding when administered peripherally. Intake of food and water, excretion of feces and urine, and body weight were recorded during the two days prior to and after the injections.

The findings reported lead us to speculate that calcitonin has a specific physiologically relevant action in the CNS. We suggest this because: (i) inhibition of feeding by intracerebral calcitonin mimics the effects of peripheral injections, in a dosage range that is on the order of the proportion of the weight ratio of brain to total body mass. (ii) The dosages of intracerebral calcitonin that inhibit feeding are smaller than the minimum dosages of most other peptide hormones of extra-neuronal origin (cf. 14), such as ACTH, angiotensin II, and the enkephalins, that have previously been shown to induce behavioral alterations when administered into rat cerebral ventricles. (iii) Inactivation of calcitonin by treatments expected to alter the characteristics of calcitonin as a peptide eliminated the ability of calcitonin to inhibit feeding. (iv) Such evidence as we have does not indicate that calcitonin inhibits feeding by producing illness or debilitation. It is therefore tempting to speculate that endogenous calcitonin is involved in the regulation of feeding and appetite, perhaps in certain specific situations, such as in infants, during lactation, or in calcium-specific hunger. However, further research will be necessary to determine whether the effects of calcitonin on feeding are merely pharmacological, or whether they mimic an effect of endogenously-secreted calcitonin.

XX. Tardive Dyskinesia

Tardive Dyskinesia (TD) is considered a major limitation to treatment with neuroleptics. There are, however, controversies regarding its incidence, etiopathology, course, prevention and treatment.

Twenty-one patients over the age of 50, who were admitted to New York (Westchester division) during a one-year period, and who had tardive dyskinesia were followed for a mean of 13 months. Neuroleptics and antidepressants were discontinued for at least three months. Dyskinetic symptoms abated in 12 patients, and persisted in 9. Discriminant function analysis showed that the persistent and reversible dyskinesia groups could be clearly separated and that the one best discriminator was the number of drug-free intervals. The persistent dyskinesia group had significantly longer neuroleptic treatment and greater number of drug interruptions than the reversible dyskinesia group. Our findings raise doubts about the commonly recommended practice of frequently interrupting long-term drug treatment, at least in patients who are otherwise predisposed to develop tardive dyskinesia.

Tardive dyskinesia has been suggested that TD may be associated with structural damage in striatal area. Because of its reportedly increasing incidence, and irreversibility in some cases, there is concern about medicolegal aspects of TD. Yet, the few published studies on postmortem and antemortem neuropathologic findings in TD have yielded inconclusive and conflicting data. We wished to compare computerized tomography (CT) scans of patients with severe and long-lasting TD with those of three groups of patients: (A) Non-TD psychiatric controls matched for age, sex, race, primary psychiatric diagosis, and neuroleptic and other treatment history. Differences between CT scans of this group and TD group might suggest some "risk factors" for development of TD. (B) Huntington's chorea patients. A number of clinical similarities between TD and Huntington's chorea have led to a suggestion that these two movement disorders may have similar underlying neuropathology (caudate atrophy). (C) Age-matched normal controls as reported in literature.

We screened inpatients from two female geriatric units of a psychiatric hospital. A diagnosis of TD was made by two psychiatrists independently using strict criteria (Jeste et al., Arch. Gen. Psychiat., in press). Severity of TD was rated on standardized rating scales. Thorough psychiatric, neurologic, dental and other physical evaluations were done, and detailed history of past treatments obtained. CT scans were performed with EMI scanner on 12 patients with severe TD (duration many months to several years), and 12 matched psychiatric controls. Patients' mean age was 68.6 and mean duration of hospitalization 35.5 years. Most patients were chronic schizophrenics. CT scans (8 to 12 cuts per subject) were read "blind" by two persons for signs of cortical atrophy (Huckman's criteria), ventricular enlargement (Hahn and Huckman's criteria), caudate atrophy (bifrontal: bicaudate ratio), cerebellar atrophy (Allen's criteria) and focal abnormalities. These scans were also compared to 42 scans of patients with Huntington's chorea done earlier.

No specific differences were found between TD patients' scans and those of matched psychiatric controls. TD was not associated with caudate atrophy, unlike Huntington's chorea. Interestingly, 85% of our patients over 60, with long-term neuroleptic treatment (both in TD and non-TD groups) showed cortical atrophy and/or ventricular enlargement. By contrast, Huckman et al. (1975) found normal CT scans in 40% non-demented persons over 60. Further work is needed before interpreting this.

The above findings, which are a part of an ongoing study, indicate a need for a different look at neuropathology of TD.

We are presently conducting a series of animal experiments to test the effect of drug interruptions on a model of tardive dyskinesia. We studied two aspects of this condition - prevention of and susceptibility to TD in an animal model. The model involved demonstration of behavioral supersensitivity to amphetamine following long-term oral administration of haloperidol to rats. The results indicate that (I) intermittent administration of haloperidol does not retard development of supersensitivity and (II) initial low responders to amphetamine develop greater percentage increase in activity counts in response to amphetamine following chronic haloperidol treatment than do initial high responders. These findings suggest two clinically important and testable hypotheses: (I) intermittent neuroleptic treatment may not prevent TD, and (II) initial amphetamine response may predict susceptibility to TD.

XXI. HLA Antigens

A survey of 14 published studies found no consistent association between HLA antigens and schizophrenia. Since the studies lacked both a shared diagnostic system or biological criteria, an investigation was undertaken using Research Diagnostic Criteria and CT scan findings. Typing for HLA antigens at loci A, B and C was carried out on 126 patients. Amongst 64 Black schizophrenic patients there was an increase of HLA-A_2 that remained significant even after correcting for the number of antigens studied. When the patients for whom CT scans were available was divided according to the presence or absence of evidence of brain atrophy there was an increase of A_2 with black schizophrenic patients without atrophy (70% vs 30) that remained significant after multiplying by the number of antigens studied but there was no significant increase of A_2 on those with atrophy. Similar trends held for the white population but they failed to reach significance. The need for HLA studies on biological defined groups of schizophrenics is stressed.

Several diseases have been shown to be associated with specific human leukocyte antigens. While there have been a number of reported associations between the HLA system and schizophrenia, none of these has been consistent. This lack of consistency could be for a number of reasons including: 1) differences in diagnostic systems and 2) biological differences amongst schizophrenics.

XXII. Laterality

On the basis of a study of handedness in monozygotic (MZ) twins at least one of whom was schizophrenic we hypothesized the existence of a subgroup of schizophrenic patients characterized by anomalous lateralization and a milder form of illness. To test this hypothesis we examined laterality in a group of schizophrenic patients.

Our sample consisted of 66 in-patients at Saint Elizabeths Hospital, Washington, D.C. who met Research Diagnostic Criteria (RDC) for schizophrenia or schizo-affective psychosis. The schizophrenic patients were further subdiagnosed into chronic (present episode of at least one year duration or incomplete recovery from previous episodes) and acute (present episode less than one year or complete recovery from previous episodes) by a rater unaware of the patients laterality. The patients were tested for laterality on a 12 item test. Those who used their right eye, hand or foot on 9 or more items were considered right handed, the others were considered not right handed (left handed or ambidextrous). Also a subgroup of 55 were given the Torque Test which consists of drawing 3 circles with each hand. Those who drew a circle in the clockwise direction with either hand were said to have Torque. Showing Torque is thought to be a left-handed characteristic and in behaviorally disturbed children has been shown to be associated with a figure diagnosis of schizophrenia.

The results indicate that anomalous lateralization, as determined either by a battery of tasks or by the Torque test, appears to be associated with a milder form of schizophrenia. This is similar to the findings of Lishman and McMeekan who found an increase in left handedness in schizoaffective psychoses but not "true" schizophrenia. It is also in keeping with our hypothesis that there exists a subgroup of schizophrenic patients with a milder form of illness who show anomalous lateralization. As a corollary, these results suggest that in severely ill schizophrenics laterality disturbances may not be more prevalent than in the general population.

If our interpretation is correct it has several implications. First, it suggests that the proportion of left handedness in a group of schizophrenics will vary depending on the percentage of milder forms of schizophrenias included in this sample. It is also possible that anomalous lateralization is important in the etiology of some schizophrenias. A study of MZ twins with schizophrenia shows that in twinships with at least one left handed twin the left handers tend to suffer from a mild schizophrenia and the right handers are not schizophrenic. This suggests that left handedness may, in some individuals predispose to schizophrenia. Also reversed neuroanatomical asymmetries on computed tomography scans, (another measure of anomalous lateralization) have been found with increased frequency only in those schizophrenic patients who show no evidence of brain atrophy. This supports the notion of etiological heterogeneity of schizophrenia and the possibility that anomalous lateralization is etiologically significant in some schizophrenic patients.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01336-08 SMRC

PERIOD COVERED
October 1, 1978 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Longitudinal Studies of Schizophrenic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas D. Reynolds	Chief, Section on Psychophysiology of Schizophrenia	SMRC
OTHER:	Ivan Waldman	Psychologist	SMRC

COOPERATING UNITS (if any)

none

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing collection of daily behavior of 11 schizophrenics and two organic patients from WAW-4. Purpose is to explore evidence for homeostatic regulation in such data and to characterize such regulation mathematically and seek in basis of differentiating (1) clinical groups and (2) changes occurring with treatment changes in individual patients. Controlling hypothesis is that behavioral steady states are more difficult for schizophrenics to achieve and our basis of his progressive withdrawal. Data suggests hebephrenics exhibit most marked behavioral cycles. Focus will be on comparing effects on varying psychotropic drug dosage on time-series parameters (phenothiazines and lithium). Review of past data confirms spectrum peaks all hebephrenic records, none for paranoid and catatonic patients. Phase plane analysis suggests unitary theoretical model for all data (modified volterra scheme).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To obtain a quantitative description of behavior which permits quantitative comparison of a variety of theoretical models. Governing the regulation of higher common excitatory states.

Methods Employed:

Behavioral notes are made 30 times daily on each of 13 chronic patients (11 schizophrenics, 2 organics for comparison purposes) on Ward 4 of the William A. White Building of Saint Elizabeths Hospital. The unit datum is a percentage of daily (or weekly) check-times positive for a given behavior (e.g., pacing, finger movement, talking, etc.). The resulting time-series is examined in a variety of ways to evidence for non-random components. Both discrete time series approaches and stimulation techniques utilizing differential equations are being applied to the data.

Major Findings:

Behavioral cycles have been identified in 5 of the patients on Ward 4. Power spectra and auto-regressive schemes.

Preliminary data suggest that lithium eliminates a six-day cycle in one patient, modifies it in two patients. Librium has an additional flattening effect on one five-day cycle when added to lithium.

Significance to Bio-Medical Research and the Program of the Institute:

While the basic purpose of this study continues to be the ultimate development of input-output modeling of human behavior in specific environments, with particular reference to a study of disturbances of regulation in the major psychoses, it would appear that the overall methodology may be applicable to the study of response to psychotropic drugs, and perhaps yield some clues as to their mode of action in functional as opposed to biochemical terms. Overall strategy controlled by the hypothesis of a homeostatis deficit in schizophrenia. Hebephrenia seens biologically separable on the basis of current data, and supports concept of central homeostatic failure in these patients.

Proposed Course:

Continuation of (1) data collection; (2) theoretical modeling; and (3) evaluation of drug responses. Current strategy is to use theoretical modeling with differential equations on all long data stretches with stable medication regimes, and examine parameter changes due to medication and/or environmental influences (e.g., participation in new treatment program without drug change.)

Publications:

Reynolds, T. D., London, W., and York, J.: Behavioral rhythms in schizophrenia. *J. Nerv. Ment. Dis.* 166: 489-499, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01337-08 SMRC

PERIOD COVERED
October 1, 1978 to September 30, 1979.

TITLE OF PROJECT (80 characters or less)
Studies on Drugs of Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Wyatt	Chief, Lab. of Clin. Psychopharmacology	SMRC
	R. C. Stillman	Research Psychiatrist	SMRC
	E. V. DeRenzo	Psychologist	SMRC
	E. Parker	Psychologist	NIAAA
OTHER:	O. Wolkowitz	Psychologist	SMRC
	P. Renault	Psychiatrist	NIDA
	H. Weingartner	Psychiatrist	APB

COOPERATING UNITS (if any)
Division of Research, National Institute of Drug Abuse; Laboratory of Clinical Science, National Institute of Mental Health; Adult Psychiatry Branch, National Institute of Mental Health.

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.85	2.5	1.35

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project continues to be determination of the mechanism of actions of drugs of abuse (particularly marijuana and alcohol) and the reasons by these drugs are used. We are also interested in ways that the understanding of drug effects can help us understand normal mental function or mental illness.

Project Description:

Objectives:

To learn about the differential effects of marijuana and alcohol on human cognition.

Methods Employed:

Subjects were chronic marijuana users, usually administered natural marijuana cigarettes or a placebo cigarette prior to the biochemical or psychological testing. Under conditions of alcohol testing, subjects were given 1 ml 95% EtOH or a placebo prior to testing. This permits the comparison of alcohol and marijuana at only one dose each, so that any findings about the effects of the two drugs would have to be in terms of profiles of action rather than comparisons along only one variable.

Major Findings:

Marijuana produced no effect on lateralization of an over-learned Posner task, but alcohol inhibited the within-day right-lateralization of the task whom by marijuana, alcohol and no-drug groups. There was a distinct right-lateralization of "same" identifications over the course of three non-consecutive days of testing, quite independent of the drug used. This right-lateralization is being studied in a group of non-drug-users. Alcohol and marijuana were compared in a consolidation task. Alcohol ingestion between study and recall was associated with higher recall than placebo; marijuana with lower.

Marijuana and alcohol were compared at single doses in a task which required the subjects to distinguish between what they had actually seen from what they had only thought about. Marijuana showed little effect on subjects' ability to make this distinction; alcohol showed a statistically significant effect on remembered frequencies for internal events.

Work in progress includes: (1) a study of the relation between visual search, immediate and long-term memory, and marijuana/alcohol; (2) the effects of marijuana land alcohol on incidental vs. intentional memory; (3) an evaluation of a simple and rapid pencil-and-paper cognitive test battery to distinguish between marijuana and alcohol intoxication; (4) additional studies to uncover alcohol's effect on consolidation.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01338-01 SMRC

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on Aging.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.J. Wyatt, Chief, Lab. of Clin. Psychopharmacology, J.C. Gillin, Deputy Chief, Lab. of Clin. Psychopharmacology, SMRC; W.B. Mendelson, Staff Psychiatrist, SMRC; D. Shore, Staff Psychiatrist, SMRC; D.R. Weinberger, Staff Psychiatrist, SMRC, M.J. Perlow, Staff Psychiatrist, SMRC; W.J. Freed, Staff Fellow, SMRC; B. Phelps, Staff Fellow, SMRC; L.M. Neckers, Staff Fellow, SMRC; D.V. Jeste, Visiting Associate, SMRC; T.P. Bridge, Staff Psychiatrist, SMRC; OTHER: J. Brodie, NIA; B. Parker, NIAAA; S. Rapport, NIA; B.J. Hoffer, University of Colorado; L. Olsen, Karolinska Institute; A. Sager; Karolinska Institute; C. Gajdusek, NINCDS; H. Weingartner, DCBR; N. Sitaram, DCBR; M. Ebert, DCBR.

COOPERATING UNITS (if any)
National Institute of Neurological Disease, National Institute of Aging, National Institute on Alcohol and Drug Abuse, Karolinska Institute, University of Colorado.

LAB/BRANCH
Laboratory of Clinical Psychopharmacology.

SECTION
Unit on Geriatric Psychiatry.

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 10	PROFESSIONAL: 9	OTHER: 1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To begin a broad based research program into the cognitive and emotional defects that occur with age.

Project Description:

Objectives:

To test existing and create new hypotheses relating to the social, psychological, cognitive and affective changes that occur with age.

Methods Employed:

The methods employed are directed at developing tools appropriate for studying older individuals.

Major Findings:

I. Drug Studies

A. Benzodiazepines

The benzodiazepines are the most commonly prescribed drugs in this country particularly in the elderly. Routine monitoring of benzodiazepine concentrations is desirable for many reasons. Patient dose requirements vary widely, which may be due to the large range of blood levels reported following the same dose. Because of benzodiazepine side effects, lethality when mixed with alcohol, and risk of physiological and psychological dependence appear dose related, patients should be maintained on the lowest effective therapeutic dose. Although threshold levels must be attained for therapeutic efficacy, excessive levels may cause clinical worsening. The principal method for measuring benzodiazepines, gas liquid chromatography, has not attained routine clinical use because of its technical complexities; also, development of a specific assay is required for each drug and its metabolites. We describe here an assay for benzodiazepines in saliva based on competition for diazepam receptor binding.

In this assay, saliva is used without any extraction or precipitation. Saliva is first centrifuged (10,000 g for 20 mins) to separate it into mucous sediment and serous supernatant. The serous supernatant is diluted by a factor of two with 50 mM Tris buffer pH 7.7 at 25°C and added directly to the assay. For the benzodiazepine radioreceptor assay, fresh rat frontal cortex was homogenized (Brinkman Polytron, setting 6 for 30 secs) in 50 volumes (w/v) 50 mM Tris buffer, pH 7.4 at 25 C. The homogenate was centrifuged twice at 50,000 g for 10 mins, with re-homogenization of the intermediate pellet in fresh buffer. The final pellet (which may be stored frozen) was resuspended in 15 volumes of freshly prepared 50 mM Tris buffer, pH 7.4 at 25 C. H-diazepam (80 Ci mmol^{-1}, New England Nuclear) was diluted to 5 nM in fresh buffer. Polypropylene 12x75 mm incubation tubes received, in order, 100λ diluted saliva (on benzodiazepine therapy or drug free), 50 3H-ligand, 50 λ drug for standard curve or nitrazepam for blanks (final concentration 1μ m) or the drug solvent buffer, and tissue suspension to 0.5 ml total volume. Final concentration of ^{3}H-diazepam was 0.50 nM. The tubes were incubated in triplicate at 0^{O}C for 45 mins and rapidly filtered under vacuum through Whatman GF/B filters with two 5 ml rinses of ice cold 50 mM Tris buffer pH 7.4 at 25^{O}C. ^{3}H-diazepam trapped on the filters was counted by liquid scintillation spectrometry after remaining overnight in scintillation vials containing Aquasol (New England Nuclear). A standard displacement curve for the drug under study was determined in the presence of equal volumes of control saliva with final concentrations of drug about one-third, three times, and the same as its IC50 value for inhibiting ^{3}H-diazepam binding under the conditions described. A log-probit plot was used to convert the displacement curve to a straight line, allowing percentage inhibition of ^{3}H-diazepam to be converted to molar drug concentration.

The absence of interference by saliva itself is apparent from experiments in which 100 λ of diluted saliva in a total assay volume of 0.5 ml reduced ^{3}H-diazepam binding by a mean ± SEM of only 1.60 ± .08%. Increasing volumes of saliva reduced binding linearly with about 45% inhibition occurring with 200 λ of diluted saliva. Saliva reduces specific and non-specific binding to the same extent, suggesting that saliva proteins bind ^{3}H-diazepam, making less available for interaction with brain membranes. The inhibition of binding by the saliva of several laboratory personnel was uniform, as was inhibition of binding by the same subjects' saliva collected on different days. Variability of inhibition of specific binding for repeated determinations of the same sample was less than 50%.

Benzodiazepines in saliva reduce the specific binding of ^{3}H-diazepam more than the small degree of inhibition caused by drug-free saliva, and the amount of inhibition of specific binding is proportional to the amount of benzodiazepine in saliva. The amount of benzodiazepine in a saliva sample can easily be determined using a standard curve of inhibition of ^{3}H-ligand binding by known amounts of the drug.

This method requires that benzodiazepines present in saliva will not inhibit non-specific (blank) binding of ^{3}H-ligand. In 14 saliva samples from patients on benzodiazepines, blank ^{3}H-diazepam binding was 231 ± 5 CPM, while non-specific binding in the presence of 12 control saliva samples was 205 ± 7 CPM. Thus, specific binding in the presence of patient saliva can be determined by subtracting non-specific binding values obtained with control saliva from total binding with patient saliva. The percentage inhibition of specific ^{3}H-diazepam binding (in the presence of control saliva) by the patient saliva (containing drug) is then calculated and compared to a standard displacement curve for determining actual benzodiazepine content.

Similar reductions in specific and non-specific binding of ^{3}H-diazepam by saliva indicates that saliva components may bind benzodiazepines making less available for membrane binding. In this assay, benzodiazepines bound to saliva components dissociate during the incubation so that total benzodiazepine levels are measured. To confirm this, 100 nM diazepam was pre-incubated for 10 mins at 37^{O}C with control saliva to allow binding to saliva proteins to occur. The saliva with diazepam was then added to a standard binding assay. The time course and extent of reduction of binding was the same for diazepam pre-incubated with saliva and diazepam dissolved in buffer. The maximum percentage lowering of binding was equivalent to that produced by 100 nM diazepam added directly to the assay with no saliva present.

Recovery was examined as follows: four different benzodiazepines were preincubated with saliva for 10 mins at 37°C to provide for binding to saliva proteins and subsequently assayed for benzodiazepine levels. Diazepam (100 nM), chlordiazepoxide (1000 nM), nitrazepam (100 nM), and oxazepam (100 nM) were all fully recovered in the benzodiazepine radioreceptor assay with respective values of 97 ± 0.20 nM (n=4), 1000 ± .07 nM (n=4), 100 ± .46 nM (n=4), and 100 ± .13 nM (n=4).

The validity of the radioreceptor assay was assessed by comparing its results with saliva levels measured by another procedure. We measured saliva benzodiazepine levels in patients treated with oxazepam; saliva oxazepam levels were measured on the same samples by gas liquid chromatography with electron capture and by saliva radioreceptor assay. We found a good agreement between the different methods. Using 100 λ of two-fold diluted saliva, the lower limit of sensitivity (7% inhibition of specific binding) is 0.14 ng/ ml, 1.50 ng/ml, 0.28 ng/ml, and 2.87 ng/ml for diazepam, chlordiazepoxide, nitrazepam and oxazepam, respectively.

In summary, the simplicity of the benzodiazepine receptor assay for saliva, as well as the ease with which saliva samples (0.2 ml each) can be collected and stored, make this assay suitable for routine clinical use in large patient populations. Saliva can be collected at home and refrigerated for later assay. The assay is selective; chlorpromazine (10^{-5}M), haloperidol (10^{-5}M), fluphenazine (10^{-5}M), desmethylimipramine (10^{-5}M), pargyline (10^{-5}M), dl-propranolol (10^{-5}M), and levallorphan (10^{-5}M) incubated with saliva failed to inhibit ^{3}H-diazepam binding more than saliva itself, suggesting that this assay can be used in patients who are being treated with a variety of other drugs besides benzodiazepines. For patients receiving more than one benzodiazepine, the concentration of each cannot be determined separately. In such cases, inhibition of ^{3}H-diazepam binding can be converted to a "diazepam equivalent" which would produce the same degree of inhibition.

An advantage of this assay is that it would detect benzodiazepine metabolites that compete for 3H-diazepam binding and which may thus be clinically active. There is evidence that a major part of diazepam's clinical efficacy is due to its metabolites desmethyldiazepam and oxazepam. Both bind to the diazepam receptor and would thus be detected in this assay.

The radioreceptor assay for benozidazepine in saliva is simpler to perform than would be a radioreceptor assay for benzodiazepines in plasma or red blood cells. We have found a high degree of inhibition of 3H-diazepam specific and non-specific binding by both plasma and red blood cell lysates. This would necessitate extra steps for further purification of plasma by deproteination and centrifugation. In the radioreceptor assay for benzodiazepines in saliva this is not necessary. Dilute serous saliva is added directly to the assay without further extraction or purification. Another possible advantage is that saliva benzodiazepine levels may more directly reflect intracellular drug levels, and thus may correlate more closely with clinical efficacy.

B. Memory

Studies conducted in the Unit on Sleep Studies in collaboration with Drs. Ebert and Weingartner at the Clinical Center suggest that normal volunteers learn more rapidly when treated with arecoline, physostigmine, or choline. Conversely, they learn less well when treated with scopolamine. These results indicate that learning and memory are fascilitated by the cholinergic system. Studies are currently underway, on the effects of arecoline and lecithin (a sympathetic precursor of acetylcholine) in patients with dementia.

C. Dementia Reduction of 5-Hydroxytrytophan

Markedly decreased levels of 5-hydroxytryptamine (5-HT), as assayed by high pressure liquid chromatography, have been found in brain tissue and blood of mice with advanced scrapie disease compared to control mice. Normal levels in brain were 340 ng 5-HT/g while less than 20 ng/g could be detected in scrapie affected mice. Normal levels in blood were 769 ng/ml versus 462 ng/ml in affected animals. In hamsters, blood levels in uninoculated age matched animals were 258 ng/ml, and in animals sham inoculated with normal brain they were 319 ng/ml, whereas in scrapie affected animals they were 118 ng/ml.

Whether this greater than ten-fold decrease in 5-HT levels in brain and two-fold decrease in blood indicates a primary loss in synthetic ability, an enhanced catabolism, or a new binding activity for 5-HT secondary to the pathological changes is as yet unknown. Analysis of 5-HT can, however, be used to follow the course of infection in laboratory animals and the natural diseases.

D. Aluminum

Several investigators have noted that patients with senile dementia have high aluminum concentrations in the cerebral cortex and other tissues and suggest that aluminum may be a causative agent in certain dementias of old age. We have been taking blood samples from patients with senile dementia and age-matched controls. Serum samples will be sent to U. of Va. Med. Coll. for aluminum assays and to San Pedro, Calif. for parathyroid hormone (PTH) determinations (since PTH causes aluminum accumulation in cerebral cortex). Samples will be sent out on a blind basic within the next month. We have also begun to collect CSF as approved to determine aluminum levels- these will be kept frozen until enough have been collected to send for analysis. We are also in the process of collectiing hair samples for blind analysis of mineral levels by Mineralab in Hayward, California. If we can show differences in aluminum levels and/or distribution in senile dementia this could have implications for both diagnosis and treatment.

E. Paraphrenia

American psychiatry has traditionally adopted the view that late life paranoid states are rare and that paraphrenia (late onset paranoid schizophrenia) should not be recognized as a diagnostic entity. A review of world literature strongly suggests that this group of patients should not be discussed so casually.

Multiple studies outside the United States concur that late life paranoid states or paraphrenia represent about 10% of all first admission psychiatric patients over the age of 60. Furthermore, there is evidence that this disorder has premorbid characteristics that distinguish it and that there is a genetic determinant to this particular disease. Diagnostic reliability for paraphrenia can be demonstrated. In fact, cross national studies between the United States and the United Kingdom indicate that United States psychiatrists most often misdiagnose hostile, belligerent, paranoid patients as organic rather than functional. This assumes great importance when one considers that nearly universal treatment response to phenothiazines among these patients that is reported in the European literature. Finally this group of patients is of interest for its research potential. Both symptomatically and genetically this group appeared to be intermediate between younger chronic schizophrenics and the population at large. As such, they can provide opportunity to learn, for example, about the timing of onset of psychosis, the effects of aging upon psychoses, or the relationship of paranoid psychoses- personality integration. Most importantly, evidence would suggest that this group deserves diagnostic recognition in order to provide them access to treatment which has proven so efficacious for them.

F. Brain Grafts

Many neurological disorders are associated with degeneration of discrete populations of neuronal elements. Parkinson's Disease, for example, manifested primarily by abnormalities of movement and posture, is characterized by neuronal loss and gliosis in the dopamine-containing zona compacta of the substantia nigra. Neurochemically, the syndrome is characterized by decreased concentrations of dopamine (DA) and its principal metabolite, homovanillic acid (HVA) in the neo- and paleo-striatum (caudate, putamen, globus pallidus) and substantia nigra.

Current therapeutic approaches to Parkinson's Disease involve peripheral administration of L-DOPA, a precursor for dopamine, and dopamine-like agents. In practice, despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or associated with severe untoward side effects. Many of these difficulties may result from: (i) use of a precursor rather than the transmitter substance itself; (ii) generalized distribution of the drugs in brain rather than focal release from terminals in discrete areas; and (iii) absence of the physiological mechanisms which normally regulate transmitter release from dopaminergic terminals. We have attempted to circumvent these problems by using central nervous system grafts as a source of dopamine in an animal model of substantia nigra degeneration.

We now report that fetal rat substantia nigra neurons can be homologously transplanted in juxtaposition to dopamine-denervated host adult caudate. The grafts proliferate extensively *in situ* and establish a behaviorally appropriate functional input to the denervated caudate.

As an experimental model, we chose the rat with unilateral destruction of the substantia nigra DA system by 6-hydroxydopamine (6-OHDA). Within one week following an unilateral injection of 6-OHDA into the substantia nigra, DA-containing cells in that site, and their axonal terminals in the corpus striatum degenerate; DA concentrations in the corpus striatum are greatly reduced. To compensate for the loss of DA, it is postulated that striatal neurons develop increased sensitivity to DA. This "supersensitivity" is manifested as: increased numbers of DA receptor sites and increased DA-stimulated adenylate cyclase activity, a decrease in the threshold of caudate neurons to the inhibitory effects of iontophoretically applied DA agonists, and a pronounced rotation contralateral to the lesion, after systemic administration of the DA receptor agonist, apomorphine. If a brain graft developed a DA input to the denervated caudate nucleus, we postulated that the supersensitivity, manifested as apomorphine-induced rotation, would be reduced.

Unilateral destruction of DA cell bodies in male Sprague-Dawley rats (150-160 gm) was accomplished using injection of 6-OHDA into the right substantia nigra and ascending DA axon bundle. After 2-4 months, the rats were placed in a rotometer and given a subcutaneous injection of apomorphine hydrochloride (0.1 or 0.25 mg/kg body weight). The number of clockwise and counterclockwise turns was recorded separately for each animal at 5-minute intervals for 40 minutes. Testing was repeated twice a week for 3-4 weeks.

Rats that had stable patterns of rotation were subjected to grafting operations. Pieces of fetal ventral mesencephalon containing the substantia nigra (SN) or adult sciatic nerve were dissected out and injected into the lateral cerebral ventricle ipsilateral to the 6-OHDA lesion. Baseline turning rates were not statistically different for the animals in SN and sciatic nerve implant groups prior to transplantation ($p<0.50$, F test).

Four weeks after grafting, animals were again tested for rotation in response to the same dose of apomorphine (twice a week for 3-4 weeks). By and large, most animals did not rotate as much after grafting as before, but the reduction in turning was significant greater for animals grafted with SN tissue than for animals with sciatic nerve implants. Data were analyzed statistically in terms of the ratios of the mean turning rate prior to transplantation, for each of the eight 5-minute segments of the 40-minute testing session. A two-way analysis of variance (groups x measures) revealed a significant main effect of the type of graft ($F=5.36$, $dF=1$, p 0.03). The main effect of measures (comparing 5-minute segments) was not significant.

The caudate nucleus and periventricular tissue of 12 of the 29 animals studied were examined using Falck-Hillarp fluorescence histochemistry and a double-blind protocol. Five of these animals had SN grafts and had at least a 70% reduction in their rotatory response to apomorphine (R animals). Four of 12 had SN grafts but less than 10% reduction in rotatory response to apomorphine (NR animals). The remaining three had sciatic nerve grafts (controls).

Although the sciatic nerve fibers degenerated after grafting, the histochemical studies revealed excellent survival and growth of the ventral mesencephalic SN grafts. Of 30 monoamine-containing implants examined in 9 animals, all but one showed large numbers of catecholamine and serotonin containing neurons with a morphology typical of adult substantia nigra and raphe, respectively. The catecholamine and serotonin containing cell bodies formed one or more dense clusters in the graft which were clearly separate. Monoamine containing fibers proliferated extensively within the grafts and invaded the adjacent caudate in all nine animals with SN implants. The catecholamine fibers that grew into the denervated caudate formed a network only in the part of the caudate adjacent to the graft. A similar ingrowth was seen into the lateral septum. However, no monoamine containing fibers invaded the corpus callosum, even at points of attachment of the graft. Monoamine containing fibers also formed dense sheets of fibers within the ependyma of the lateral ventricle. For the most part, anteriorly placed grafts formed well-demarcated structures in the lateral ventricle opposed to or fused with the medial surface of the caudate and lateral surface of the septum. Some deeper grafts lay interstitially, fused with the host brain tissue. Transplants that were placed more posteriorly in the lateral ventricle were usually found adjacent to or fused with the fornix and dorsal alveus of the hippocampus. There was little or no evidence of any scaring or pathological disturbances of the host striatum.

It was of great interest to compare the histochemical properties of grafts in animals that showed reduced turning after grafting with those that did not. Both groups had approximately equal mean numbers of catecholamine-containing cell bodies per animal and proliferating fibers in the graft. Moreover, the ingrowth of fibers into the adjacent caudate was approximately equal in both groups. The major difference in the two groups was in the degree of caudate denervation produced by the 6-OHDA injection. The degree of initial 6-OHDA denervation could be estimated by histochemical examination of the host's caudate, remote from graft, and by counting the remaining dopamine-containing cells in the host's substantia nigra. All R animals were virtually totally denervated. Every NR animal had some residual endogenous DA-containing cell bodies in the nigra and weak to very weak diffuse endogenous DA fluorescence in large areas of the caudate. A second difference was in the location of the graft. NR animals tended to have a more posterior graft localization within the ventricle, while R animals had a more anterior placement.

From the above studies, we conclude the following: (1) The grafts of fetal SN to the lateral ventricle adjacent to the caudate nucleus of the rats survived and proliferated. All but one of the grafts examined survived without rejection for at least two months; (2) grafting of the fetal SN reversed, to some degree, the behavioral changes associated with destruction of the host nigro-striatal system; (3) this recovery was associated with the growth of DA-containing cells and fibers in the grafts and into host brain tissue. However, from the data reported here, the functional recovery cannot be ascribed strictly to re-innervation of the caudate; release of DA and subsequent diffusion from the grafts into the caudate is another possibility. (4) Incomplete destruction of the endogenous DA input, although sufficient to induce "behavioral supersensitivity", may antagonize reversal of the supersensitivity by a subsequent SN graft. (5) The small reduction in turning seen in control animals may result from a disturbance in caudate function secondary to grafting procedure, although histological examination did not reveal evidence of caudate injury.

Although numerous studies have demonstrated (i) the regeneration of mammalian nervous tissue following injury, (ii) that various parts of the mammalian central nervous system can be grafted to peripheral tissue, and (iii) that non-nervous tissue can be grafted to the brain, there have been few studies demonstrating that central nervous system tissue could be grafted to and survive in the brain or spinal cord of another animal. In some studies where the grafted nervous tissue has been placed into host brain areas, fiber proliferation and synapse formation have been demonstrated. This is, to our knowledge, the first demonstration, however, that the grafting of mammalian brain tissue from one animal to another elicits an alteration in the behavior of the recipient animal consistent with the normal function of the grafted tissue.

Parkinson's Disease has a wide variety of signs and symptoms; not all of these are reliably improved by current therapy. Our data show that catecholamine-containing tissue, implanted in proximity to the caudate survives, proliferates and can produce improvements in lesion-induced motor abnormalities. While clearly much additional research is required to establish the safety and long term stability of grafts, there is a possibility that such a local catecholamine source could offer the patient with Parkinson's Disease a more physiological treatment than is presently available. In this context, it is important to explore autografts and/or homografts of peripheral catecholamine-containing tissues, such as adrenal medulla. If further studies can establish the efficacy and safety of this approach for therapy, it may eventually be possible to employ techniques similar to those that we have used here in the treatment of other neurological disorders where the loss of nerve cells is reasonably well circumscribed.

Publications:

1. Rosenblatt, J.E., Bridge, T.P. and Wyatt, R.J.: A novel method for measuring benzodiazepines in saliva. Communications in Psychopharmacology, in press.

2. Perlow, M.J., Freed, W.J., Hoffer, B.J., Seiger, A., Olson, L., and Wyatt, R.J.: Brain grafts reduce motor abnormalities produced by destruction of the nigro-striatal dopamine system: Behavioral and histochemical evidence. Science 204: 643-647, 1979.

3. Bridge, T.P., Cannon, H.E., and Wyatt, R.J.: Burned out schizophrenia: Evidence for age effect on schizophrenic symptomatology. Journal of Gerontology 33: 835-839, 1978.

4. Jeste, D.V., Gillin, J.C. and Wyatt, R.J.: Serendipity in biological psychiatry--A myth? Archives of General Psychiatry, in press.

5. Jeste, D.V. and Wyatt, R.J.: Tardive dyskinesia. Psychiatric Annals, in press.

6. Jeste, D.V. and Wyatt, R.J.: In search of treatment for tardive dyskinesia: A review of the literature. Schizophrenia Bulletin, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01339-04 SMRC

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Eye Tracking in Schizophrenic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas D. Reynolds	Chief, Section on Psychophysiology of Schizophrenia	SMRC
OTHER:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eye tracking of schizophrenic and normal controls is being explored. Pilot study suggests simple attentional deficits, correctable by making stimulus situation more interesting.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To attempt replication of reported finding of defective eye-tracking in schizophrenia.

Methods Employed:

Electrooculogram is recorded while subject follows a pendulum movement. Various stimuli are affixed to pendulum bob.

Major Findings:

Over 40 patients have been studied. Eye-tracking in schizophrenia most markedly improved by affixing picture of a face on the bob. Similar findings have been reported, but our data suggest schizophrenia tracking more closely approximate normal tracking than hitherto reported.

Significance to Bio-Medical Research and the Program of the Institute:

Utility of eye-tracking as a genetic marker rendered doubtful. However, as a sensitive technique for recording attentional deficit and the variables which influence the latter, the tracking task may be ideal. It will be pursued with more practical goals in mind (i.e., a technique for monitoring treatment response).

Proposed Course:

We expect to continue to use apparatus as part of functional test battery used to monitor treatment response.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01489-06 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of gene expression in neuroblastoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Hollenbeck	Pharmacologist	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found that activation of protein kinase by 8 bromo adenosine 3',5'-cyclic monophosphate (8 Br-cAMP) in cultured neuroblastoma cells (NB) is followed by preferential phosphorylation of nuclear nonhistone chromosomal proteins and the induction of tyrosine hydroxylase (TH). Our experimental results suggest that TH induction in NB may be preceded by a modulation of gene expression through translocation of protein kinase from cytosol to nucleus and phosphorylation of nuclear nonhistone protein.

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01492-07 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Properties of monoamine oxidases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. H. Neff	Chief	SMRP-B	NIMH
Other:	R. J. Wyatt	Chief	SMRC	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to survey the types and activities of monoamine oxidase (E.C. 1.4.3.4) in autopsy material from individuals with various psychiatric disorders.

PHS-6040
(Rev. 10-76)

Project Description:

The monoamine oxidases (E.C. 1.4.3.4:MAO) are enzymes which participate in the inactivation of biogenic amines. Drugs that inhibit MAO are used clinically as antidepressants and antihypertensives. The identification of Type A and B MAO (see M-SMR-P-12, 72-72) has prompted studies to define the role of these enzymes. Our objective was to continue our survey of the types and activities of MAO in autopsy material from individuals with various psychiatric disorders.

MAO activity was determined using serotonin (a substance for Type A enzyme) or phenylethylamine (a substrate for Type B enzyme) as substrate.

To date we have processed about 30 human liver autopsy samples from both control and schizophrenic subjects and found no significant difference between the two groups.

Monoamine oxidase activity is low in blood platelets from schizophrenics and the low activity may have a heritable component. We are attempting to determine if enzyme activity in various tissues other than platelets is low in various psychiatric disorders.

MAO inhibitor drugs are used to treat hypertension and depression. Our studies may lead to a better understanding of depression and hypertension and how drugs such as MAO inhibitor might alleviate these disorders. They may also provide information on the etiology of some mental illnesses.

We will continue our investigation of human autopsy material for Type A and Type B monoamine oxidase activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01500-07 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Indolealkylamines in the central nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. Lackovic	Visiting Associate	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective is to determine if spinal nerve roots contain serotonin and 5-hydroxyindoleacetic acid. The presence of these substances would suggest that indolealkylamines play a role in processing of information in the peripheral nervous system.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective is to determine whether serotonin might play a role in the processing of information in the peripheral nervous system. In our initial studies gas chromatographic-mass spectrometric methods were used to identify the presence of serotonin and 5-hydroxyindoleacetic acid in spinal nerve roots.

Spinal nerve roots were dissected from bovine and rat spinal cord and assayed by multiple ion detection gas chromatography-mass spectrometry.

Serotonin and 5-hydroxyindoleacetic acid were positively identified in dorsal and ventral nerve roots by multiple ion monitoring. Dorsal roots contained more serotonin than ventral roots.

Serotonin and substance P are found in the same neurons in many regions of brain. Our finding that serotonin is found in the spinal nerve roots suggest that it might be found in the same neurons that contain substance P in the spinal nerve roots. Abnormal serotonin metabolism has been implicated in several psychiatric and sleep disorders. These disorders might be related to a biochemical failure of the serotonergic system of brain. The spinal nerve root serotonin system may provide a simple biochemical model for studying serotonin-substance P neurons and to evaluate the pharmacology of drugs.

An extensive study will be undertaken to determine the origin and projections of the spinal nerve root serotonin system.

Publications:

Neckers, L. M., Neff, N. H., and Wyatt, R. J.: Increased serotonin turnover in striatum following an injection of kainic acid: Evidence for neuronal feedback regulation of synthesis. Naunyn-Schmiedebergs Arch. Pharmacol. 306: 173-177, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01503-05 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pharmacological studies of acetylcholine turnover: Control of septal-hippocampal pathway

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Brunello	Visiting Fellow	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	P. L. Wood	Guest Worker	SMRP	NIMH
	A. Tagliamonte	Visiting Scientist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Muscimol, β-endorphin and apomorphine reduce the turnover rate of acetylcholine in hippocampus by an action in septum. Bicuculline injected intraseptally will reverse these effects suggesting the presence of septal GABAergic interneurons. Intraseptal (lateral) kainate reduces septal dopamine stimulated adenylate-cyclase and glutamic acid decarboxylase but not hippocampal choline acetyltransferase. Intrahippocampal kainate destroys hippocampal pyramidal cells and reduces high affinity glutamate uptake in septum without affecting hippocampal turnover rate of acetylcholine.

Decortication reduces turnover rates of acetylcholine and γ-aminobutyric acid in striatum. Intrastriatal kainate also reduces acetylcholine turnover in striatum. These data suggest that the cortical input to the striatum involves excitatory glutamatergic neurons modulating both GABAergic and cholinergic functions.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this study was to increase our knowledge on the neurochemistry of the local neuronal circuitry regulating the septal-hippocampal cholinergic pathway. Moreover we have wondered on the role of the cortical-striatal glutamatergic pathway.

The methods for determining the concentration and turnover rate of acetylcholine were described in Project MH 01509-08 SMRP-1976.

Injection of the GABA agonist muscimol (0.88 nmole) into the septum decreases the hippocampal turnover rate of acetylcholine. The GABA antagonist, bicuculline has no effect. The lack of action by bicuculline may suggest that the GABA interneurons do not exert a tonic influence on the firing of hippocampal cholinergic neurons. However, bicuculline, blocks the inhibition elicited by muscimol, β-endorphin or apomorphine on the hippocampal cholinergic system. This would suggest that both dopamine and β-endorphin exert their actions on acetylcholine turnover in hippocampus via the GABAergic interneurons in the septum. Kainate (0.5 μg; 15 d), L-nuciferine (20 μg; 30 min), benztropine (2 μg; 30 min), and strychnine (5 μg; 30 min) injected intraseptally and DL-α-aminoadipate (20 μg; 30 min) injected intraventricularly are without effect on the turnover rate of acetylcholine in the hippocampus. These preliminary studies raise doubts concerning the involvement of glutamate, aspartate or glycine in the control of the septal hippocampal cholinergic pathway.

Septal dopamine stimulated adenylate cyclase activity decreases sharply, together with glutamic acid decarboxylase activity 5-10 days after the injection of kainic acid (0.15 μg at each of two locations within the lateral septum). Under these conditions choline acetyltransferase in the ipsilateral hippocampus is unaffected. Since GABAergic interneurons of the lateral septum can be destroyed with no change to the cholinergic perikaria of the medial septum, many questions concerning the control of the cholinergic septal-hippocampal pathway by septal GABAergic interneurons can be answered.

Kainic acid has been used to lesion the hippocampal neurons in an attempt to study the transmitter involved in the feedback regulation of the septal cholinergic neurons. Injection of kainic acid results in a massive destruction of hippocampal pyramidal cells which is associated with a marked reduction in the high affinity uptake of glutamate in the ipsilateral septum. However, there is no effect on the hippocampal turnover rate of acetylcholine. These results suggest that glutamatergic feedback innervates the lateral septum but does not directly synapse with the cholinergic neurons of the medial septum.

Cortex-Striatum

Interruption of the cortico-striatal glutamatergic pathway by decortication results in significant decreases in the turnover rates of acetylcholine and γ-aminobutyric acid of striatum. These data support the hypothesis that the cortical input to the striatum is excitatory and acts to modulate cholinergic and GABAergic function in this nucleus. Studies of kainic acid induced lesions of the striatum also indicate that most striatal cholinergic interneurons receive a glutamatergic input and that multiple injections of kainic acid are required to destroy this large population of interneurons.

It has been proposed that alterations in the dynamics that regulate the transmitter function at the synaptic level may be involved in various forms of mental disease. Since neuronal activity is regulated by the rates of release and inactivation of transmitters, including acetylcholine, the study of the turnover rate of acetylcholine in various synaptic areas of the rat brain associated with available anatomical and histological information is a tool to study the functional role of cholinergic innervation in various brain structures of the limbic system. The frontal cortex and hippocampus are two areas of the brain which are closely associated with expressions of emotions, learning and cognitive processes. Cholinergic neurons are known to project to these areas and a study of the specific neurotransmitter control of the cholinergic neurons projecting to these areas may yield information that can be used in the design of drugs for the treatment of mental disease.

We shall continue to use local and intraventricular injections of the specific agonists and antagonists to determine the transmitter control of cholinergic dynamics. Following destruction of lateral septal GABAergic interneurons we shall study how such lesions affect the activity of the septal-hippocampal cholinergic pathway.

Publications:

Moroni, F., Malthe-Sørenssen, D., Cheney, D. L., and Costa, E.: Modulation of acetylcholine turnover in the septal-hippocampal pathway by electrical stimulation and lesioning. Brain Res. 150: 333-341, 1978.

Robinson, S., Cheney, D. L., Moroni, F., and Costa, E.: Acetylcholine turnover in specific brain areas of rats injected with various antidepressants. In Garattini, S. (Ed.): Depressive Disorders. Stuttgart, Germany, F.K. Schattauer Vorlag, 1978, pp. 129-139.

Cheney, D. L., Robinson, S. E., Malthe-Sørenssen, D., Wood, P. L., Commissiong, J. W., and Costa, E.: Regulation of the cholinergic septal-hippocampal pathway: Role of dopaminergic septal afferents. In Dumont, C. (Ed.): Advances in Pharmacology and Therapeutics. Vol. 5, Neuropsychopharmacology. Oxford and New York, Pergamon Press, 1978, pp. 241-250.

Robinson, S. E., Cheney, D. L., and Costa, E.: Effect of nomifensine and other antidepressant drugs on acetylcholine turnover in various regions of the rat brain. Naunyn-Schmiedebergs Arch. Pharmacol. 304: 263-269, 1978.

Wood, P. L., Peralta, E., Cheney, D. L., and Costa, E.: The turnover rate of ACh in the hippocampus after lesion of hippocampal pyramidal cells with kainic acid. Neuropharmacology 18: 519-523, 1979.

Cheney, D. L., Robinson, S. E., Malthe-Sørenssen, D., Wood, P. L., and Costa, E.: Regulation of hippocampal cholinergic neurons by dopaminergic synapses in septum. In *Catecholamines: Basic Clinical Frontiers*, 4th Symposium of Catecholamines, Asilomar paper, in press.

Robinson, S. E., Cheney, D. L., and Costa, E.: The regulation of hippocampal cholinergic neurons by noradrenergic mechanisms. In *Catecholamines: Basic and Clinical Frontiers*, 4th Symposium of Catecholamines, Asilomar paper, in press.

Robinson, S. E., Malthe-Sørenssen, D., Wood, P. L., and Commissiong, J. W.: Dopaminergic control of the septal-hippocampal cholinergic pathway. *J. Pharmacol. Exp. Ther.* 208: 476-479, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01505-06 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Acetylcholine γ-aminobutyric acid turnover rates: Anesthetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	A. Revuelta	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)
S. H. Ngai
Department of Anesthesiology, Columbia University,
New York, N.Y.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nitrous oxide (inspired 76%; 45 min) did not alter the concentration or turnover rate of acetylcholine in any of the brain areas studied. Pentobarbital (65 mg/kg; i.p.) reduced the turnover rate and increased the levels of acetylcholine in cortex, hippocampus and diencephalon but not in striatum. The local anesthetics, lidocaine and cocaine (100 mg/kg; s.c.; 39 min), did not change the steady-state concentrations of acetylcholine. Lidocaine decreased the acetylcholine turnover rate in the cerebral cortex; whereas cocaine, which causes hypermotility, increased the acetylcholine turnover rate in the cerebral cortex and the diencephalon but not in the striatum or the hippocampus.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the mechanisms that regulate the function of cholinergic and γ-aminobutyric acid-ergic neurons in the central nervous system following administration of gaseous, parenteral and local anesthetics.

Acetylcholine and choline concentrations and acetylcholine turnover rate were measured mass spectrometrically in different regions of the rat brain after injecting two local anesthetics, lidocaine (100 mg/kg; s.c.; 39 min) and cocaine (100 mg/kg; s.c.; 39 min) and pentobarbital (65 mg/kg; i.p.; 45 min). The same parameters were measured after adminstration of nitrous oxide (inspired 75%; 45 min). Glutamate and γ-aminobutyric acid concentrations and γ-aminobutyric acid turnover rate were measured mass spectrometrically in various brain nuclei after administration of halothane (1%; 40 min) and enflurane (3%; 40 min).

Lidocaine produced a long lasting sedation (90 min) and cocaine caused a prolonged excitation (120 min). Under these behavioral conditions lidocaine reduced the turnover rate of acetylcholine in parietal cortex but had no effect in striatum, hippocampus or diencephalon. Cocaine, on the other hand, increased the turnover rate of acetylcholine in parietal cortex and diencephalon but not in hippocampus and striatum. Cocaine produces a long-lasting CNS stimulation that is not shared by the other local anesthetics or gaseous anesthetics. Thus the increased turnover of acetylcholine induced by cocaine may be unrelated to the local anesthetic effect and may be due to its CNS stimulating action.

Pentobarbital reduced the turnover rate of acetylcholine and increased the levels of acetylcholine in cortex, hippocampus and diencephalon but not in striatum. The same pattern of change was seen in hippocampus 2 hrs after cutting the fimbria. However there is no further decrease in turnover rate in the hippocampus of rats with fimbrial lesions and treated with pentobarbital. These data are consistant with an action of pentobarbital through the cholinergic cell bodies in the septum. Further experiments will be necessary to substantiate these results.

Clarification of the influences exerted by various anesthetic agents on cholinergic mechanisms may help in understanding the role that acetylcholine plays in consciousness.

The mechanism by which pentobarbital reduces the turnover rate of acetylcholine via the septal-hippocampal pathway will be investigated. The role of the turnover rate of γ-aminobutyric acid in rat brain areas will be further studied under conditions of gaseous anesthesia.

Publications:

Ngai, S. H., Shirawawa, R., and Cheney, D. L.: Changes in motor activity and acetylcholine turnover in brain regions of rats injected with lidocaine and cocaine. Anesthesiology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01506-05 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Narcotic analgesics and the regulation of catecholaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Kumakura	Visiting Fellow	SMRP	NIMH
Other:	F. Moroni	Visiting Associate	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Morphine increases striatal dopamine turnover rate. This increase is probably due to stimulation of opiate receptors located on DA nerve terminals of striatum. Morphine injected in substantia nigra (SN) failed to change striatal DA metabolims but blocked the activation of TH by haloperidol. This action of morphine was not reversed by bicuculline. Since opiate receptors in SN are located on neurons connecting the SN with the forebrain (possibly on substance P neurons), these results suggest that intranigral morphine prevents the haloperidol-induced activation of striatal TH by interfering with a non-GABAergic (perhaps substance P) projection to SN. The role of opiate receptors located on catecholaminergic cells was studied using primary culture of adrenal chromaffin cells. These cells have measurable amounts of opiate receptors. Stimulation of these receptors with agonists decreases the release of catecholamine elicited by nicotine. The results suggest that stimulation of opiate receptors modulates allosterically the function of nicotinic and perhaps other receptors located on the chromaffin cell membranes.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the action of narcotic analgesics administered acutely or chronically on the function of various cells that release catecholamines. To establish whether narcotic analgesics interact with dopaminergic or catecholaminergic neurons at the pre- or post-synaptic receptor site we have studied changes in the NE and DA turnover, in the tyrosine hydroxylase activity (TH) of striatum and in the release of catecholamines from primary cultures of cow adrenal chromaffin cells.

1) Experiments in striatum

Analgesic doses of morphine and endorphins increased DA turnover and facilitated HVA accumulation in striatum. This effect was maintained after lesion of the cortico-striatal or striato-nigral pathway. However, after injection of morphine directly into SN (in doses of 1,2,5,10 and 20 µg of the (SO_4) salt, dissolved in 0.5 µl saline) we found no change in striatal HVA content. These results suggest that unlike the opiate receptors in striatum, the opiate receptors in SN do not mediate the morphine-induced stimulation of striatal DA metabolism, moreover they suggest that opiate receptors are located on neurons other than dopaminergic and that these are phasic neurons. To gain further clarification on this process, we examined whether intranigral morphine could inhibit the activation of striatal TH induced by haloperidol. Perhaps this activation is due to a phasic neuronal system stimulated by haloperidol. The action of haloperidol on striatal TH may be mediated via the transsynaptic regulation of DA neurons by nigral afferents, including the striato-nigral axons containing GABA or substance P. We therefore expected that if the nigral opiate receptors exert an inhibitory influence on DA neurons (either directly or indirectly), then intranigral morphine would be able to antagonize the activation of striatal TH elicited by haloperidol.

Although intranigral morphine by itself did not change the affinity of striatal TH for $DMPH_4$, it completely prevented the increase in affinity of striatal TH for $DMPH_4$ in animals treated with haloperidol. This effect of intranigral morphine was reversed by systemic or intranigral injection of the opiate-receptor antagonist, naltrexone, but was not reversed by intranigral injection of bicuculline methiodide, a GABA receptor antagonist. The specificity of the action of morphine was further supported by experiments which demonstrated that the ability of muscimol to antagonize the haloperidol-induced activation of striatal TH was reversed by intranigral bicuculline methiodide but not naltrexone.

In order to determine whether this effect could be mediated by a direct action of morphine on DA cells in SN, we measured opiate receptor binding in SN after unilateral destruction of nigrostriatal DA neurons by 6-hydroxydopamine (6-OHDA). Although 6-OHDA treatment resulted in the destruction of more than 85% of the nigrostriatal DA neurons (based on TH measurements), no significant change was obtained in the binding of ^{3}H enkephalinamide in the crude P2 fraction of the lesioned SN. In contrast, there was a marked (68%) decrease in the binding of ^{3}H enkephalinamide to SN P2 preparation from rats with surgical hemitransection of all the connections between striatum and SN. This lesion reduced both GABA and substance P content in SN by 60-85%.

In conclusion the presence of opiate receptors on various neural elements of the nigro-striatal loop supports the concept that stimulation of these receptors in selective portions of the loop will generate changes of DA metabolism that may vary from excitation to inhibition. Striatal opiate receptors located on terminals of DA neurons are probably responsible for the increase of DA metabolites, however the molecular mechanism of this increase remain unclear. In SN opiate receptors are not located on DA neurons and they are not apparently involved in a control of basal DA function. However when the DA system is pharmacologically activated by haloperidol, then activation of opiate receptors can reverse this effect. Previous work from this laboratory has shown that substance P in SN may participate in the activation of TH by haloperidol. The experiments reported here suggest that as in the spinal cord, the substance P neurons in SN may be regulated presynaptically by opiate receptors. Thus it appears that opiate peptides may represent an additional neuromodulatory factor in SN which, by activating opiate receptors, located on axon terminals secreting substance P or other excitatory neurotransmitters, can influence the activity of dopaminergic neural-striatal projections. It remains to be establish with precision the nature of the SN axon terminals that contain opiate receptors and the role of opaite on the release of transmitters from these neurons. In addition the type of opiate receptors and the origin of these opiate peptides in SN remain to be established.

2) Experiments in primary culture of adrenal chromaffin cells

The primary culture of adrenal chromaffin cells from bovine adrenal medulla offers an ideal model where to study the molecular mechanisms by which narcotic analgesics control the function of catecholamine containing cells. In fact we could show that the chromaffin cells in culture contain high affinity, saturable, stereospecific binding sites for opiate agonist and antagonist. The Bmax with the antagonist is greater than that calculated using the agonist. When these receptors are occupied by specific opiate agonist (morphine, β-endorphin and FK 33824), the number of nicotinic receptors and the amount of catecholamines released by maximal doses of nicotine are reduced. The specificity of the action of opiate receptor agonists is supported by the ability of the opiate receptor antagonists to block their effects and by the inability of the opiate receptor agonists to reduce the Ca^{++} independent release of catecholamines elicited by K^+ ions.

In conclusion opiate receptors are present in the membrane of adrenal chromaffin cells. The activation of these receptors causes a non competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data it can be inferred that when the met-enkephalin-like material present in terminals of splanchnic nerve is released by nerve impulses it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Since the Bmax for the binding of the antagonist is large chromaffin cells may contain two types of receptors, one specific for met-enkephalin and the other for β-endorphin. Beta-endorphin released into the circulation from the intermediate or anterior lobe of the pituitary can influence the function of chromaffin cells.

The interrelations between opiate agonists and catecholamine secretion are relevant to the mission of NIMH in many ways. The study we are now performing may lead to a better understanding of the molecular synaptic mechanisms involved in the reciprocal control of catecholamine and enkephalin neuronal function, two functions involved in the etiology of affective disorders.

Publications:

Guidotti, A., Moroni, F., Gale, K., and Kumakura, K.: Opiate receptor stimulation blocks the activation of striatal tyrosine hydroxylase induced by haloperidol. In Usdin, E. (Ed.): Catecholamine: Basic and Clinical Frontiers. New York, Pergamon Press, in press.

Gale, K., Moroni, F., Kumakura, K., and Guidotti, A.: Opiate receptors in substantia nigra: Role in the regulation of striatal tyrosine hydroxylase activity. Neuropharmacology 18: 427-430, 1979.

Kumakura, K., Guidotti, A., and Costa, E.: Primary cultures of adrenal chromaffin cells: Modulation of nicotinic receptors by opiate receptor agonists. Nature, submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01508-09 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Modulation by opiate receptors and antagonists of GABAergic and cholinergic function in various brain areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	E. Peralta	Guest Worker	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In rats, morphine (s.c.) and beta endorphin (i.v.t.) administration decreases the turnover rate of γ-aminobutyric acid (GABA) in the N. caudatus, but increases the turnover rate of GABA in globus pallidus and substantia nigra. The effects of morphine on the turnover rate of GABA are reversed by naltrexone. There is no dopaminergic involvement in the turnover rate of GABA. The increase of GABA turnover in globus pallidus seems to play an important role in the morphine induced catalepsy. In fact, muscimol, a GABA receptor stimulating agent, when injected bilaterally into the globus pallidus mimics morphine catalepsy. Thus, the action of morphine and beta endorphin on the turnover rate of GABA in the caudate and globus pallidus may be due to an effect on opiate receptor located in the head of the caudate nucleus. Since the GABA turnover in caudate decreases after opiate receptor administration and after surgical procedures which destroy the cortico-striatal afferents, it is possible that opioid neurons modulate the afferents to caudate and through these mechanisms modify the turnover rate of GABA.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to examine the modulation and regulation of the GABAergic neurons in various brain areas by determining if opiate receptor stimulation and dopaminergic agonist effects would in some way change GABAergic function. These studies attempt to achieve a better understanding of the synaptic link between peptidergic neurons with opiate-like activity, dopaminergic neurons, and GABAergic neurons.

The procedures and the techniques for calculating the turnover rate of GABA have been described in Projects Z01 MH 01509-06 SMRP, 1975; Z01 MH 01503-02 SMRP and Z01 MH 01508, 1978.

Qualitatively similar to subcutaneous morphine administration, intraventricular injection of beta endorphin induced analgesia and catalepsy, showed a dose dependent decrease of GABA turnover in the caudate nucleus and a dose dependent increase of the turnover rate of GABA in the substantia nigra and the globus pallidus. No changes were observed in the hippocampus or dentate gyrus in GABA content. Naltrexone antagonized the effects of both morphine and beta endorphin. Since the turnover rate of GABA may be a good biochemical index of the activity of the GABAergic neurons and since both morphine and beta endorphin stimulate the enkephalinergic receptors present in the N. caudatus and in the globus pallidus, these experiments suggest that the release of enkephalin from enkephalinergic neurons inhibit the GABAergic function in the N. caudatus and increase the GABAergic function in the globus pallidus and substantia nigra.

Since morphine increases striatal dopamine turnover rate by activating the opiate receptors located on the axons of dopamine neurons and since morphine changes the turnover rate of GABA in the N. caudatus, globus pallidus and substantia nigra, a dopaminergic effect may be involved. Both electrical stimulation of the dopamine cell bodies in the substantia nigra and intrastriatal injections of apomorphine showed no change in the GABA turnover or the GABA content in the N. caudatus. Since the metabolism of GABA in the N. caudatus is regulated by opiate receptor stimulation, the enkephalin containing neurons may regulate GABAergic interneurons transsynaptically.

In order to localize these interactions between the endogenous opiate receptors and the GABA neurons, morphine and beta endorphin were injected locally into the N. caudatus and the turnover rate of GABA was measured in the homolateral globus pallidus and substantia nigra. It appears that the caudatal GABAergic neurons interact with the pallidal GABAergic neurons due to the increase of GABA turnover in the pallidus when the output of striatal GABAergic neurons is decreased. These experiments demonstrate that morphine and beta endorphin directly interact with GABAergic neurons because they regulate GABA turnover in the N. caudatus. They also show that there is no dopaminergic involvement in GABA turnover, since there is no change in the turnover rate of GABA or GABA content after stimulation of substantia nigra and after intrastriatal injection of apomorphine.

These conclusions were confirmed by injecting naltrexone into the N. caudatus and injecting morphine subcutaneously. However, the increase of GABA turnover in substantia nigra elicited by parentally administered morphine seems to be due to an activation or inhibition of local interneurons and not to an activation of the GABAergic strio-nigral neurons resulting in an inhibition of the intrinsic GABA interneurons.

The increase in the utilization of GABA elicited by the opioids in the globus pallidus may participate in determining catalepsy because muscimol a specific GABA receptor agonist, injected directly into the globus pallidus causes a dose related catalepsy. However, the increase in GABA utilization in substantia nigra is probably involved in other actions of morphine and beta endorphin because muscimol, injected locally into the brain area failed to induce analgesia or to cause catalepsy.

Since the turnover rate of GABA in the N. caudatus decreased also after ablation of the cortex or hemitransection it is possible that the enkephalinergic neurons modulate the GABAergic striatal interneurons acting at the level of the cortico and nigra striatal pathways.

Due to the possible implications of the so called "strio-nigral loop" in severe neurological and psychiatric disorders, such as Parkinson's, Huntington's Chorea and some psychoses, biochemical studies evaluating the synaptic regulation of these pathways and the effect of drugs on these synaptic mechanisms may be of great practical interest. The study of the interactions between opioid peptides and putative neurotransmitters may clarify the brain modification leading to the development of tolerance and physical dependence and may help elucidate the pharmacology of morphine.

We plan to study the turnover rate of GABA during the development of tolerance and physical dependence and to correlate the effects of morphine and opioid peptides on GABA turnover with the behavioral effects of the two drugs. Furthermore, we plan to set up methods to investigate the effects of morphine and related peptides on other putative neurotransmitters such as glutamate, for example.

Publications:

Moroni, F., Cheney, D. L., Peralta, E., and Costa, E.: Opiate receptor agonists as modulators of gamma-aminobutyric acid turnover in the nucleus caudatus, globus pallidus, and substantia nigra of the rat. J. Pharmacol. Exp. Ther. 207: 870-877, 1978.

Moroni, F., Peralta, E., Cheney, D. L., and Costa, E.: On the regulation of gamma-aminobutyric acid neurons in caudatus, pallidus and nigra: Effects of opioids and dopamine agonists. J. Pharmacol. Exp. Ther. 208: 190-194, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01509-09 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pharmacological studies of acetylcholine turnover: Peptides and effects of stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Tagliamonte	Visiting Scientist	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	P. L. Wood	Guest Scientist	SMRP	NIMH
	N. Brunello	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
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SECTION
Molecular Pharmacodynamics

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	1.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The turnover rate of acetylcholine was studied in rats injected intraventricularly with prolactin or exposed to cold for different periods of time. Prolactin increased the turnover rate of acetylcholine selectively in the striatum and hippocampus and this effect was prevented by the previous destruction of dopaminergic pathways with 6-OHDA (injected in the A_9-A_{10} areas 10 days prior to the experiment). Exposure to cold for 1 and 4 hours selectively decreased the turnover rate of acetylcholine in the frontal cortex by about 40%. Turnover rates of acetylcholine in brain areas of rats exposed for 24 hours to cold were unchanged.

PHS-6040
(Rev. 10-76)

Project Description:

The present studies were concerned with the role of peptide receptors and the role of stress in the regulation of the CNS cholinergic system.

Experimental details have been described in Project No. Z01 MH 01509-07 SMRP 1976 and 1977. For these continuing studies on peptide control, prolactin was chosen because it can regulate the release of striatal dopamine. To administer prolactin permanent plastic cerebroventricular cannulas and intraseptal cannulas were implanted. The dopaminergic neurons located in A_9 and A_{10} were selectively lesioned by infusing 6-hydroxydopamine in the A_9/A_{10} border.

For stress experiments, to avoid any restraining procedure during infusion of stable isotopes, a chronic cannula was inserted in the jugular vein 3 days before the experiment. The cannula emerged from the skin behind the head and was long enough to allow the rat to move freely inside its cage.

Prolactin:

Striatum and hippocampus were selected as two areas where dopaminergic terminals are known to regulate the turnover rate of acetylcholine and frontal cortex and parietal cortex as two areas where dopamine has little or no control of the turnover rate of acetylcholine. Intraventricularly injected prolactin reduces the turnover rate of acetylcholine in striatum, hippocampus and thalamus but not in the two cortical areas. Intraseptal injection of prolactin reduces the turnover rate of acetylcholine in hippocampus suggesting that this polypeptide is acting on hippocampus by changing the activity of afferent neurons impinging upon the cell bodies of the cholinergic septal-hippocampal neurons. The reductions in the turnover rate of acetylcholine in hippocampus and striatum elicited by intraventricular prolactin are nullified by 6-hydroxydopamine induced lesions of dopaminergic neurons located in areas A_9 and A_{10}. These results suggest that the presence of dopaminergic neurons is required to obtain the decrease of the turnover rate of acetylcholine elicited by prolactin.

Stress:

Different stress conditions are known to affect both the neuroendocrine function and behavior of animals by changing the function of brain neurotransmitters; catecholamines have been singled out as the main mediators of stress. Since in brain various neuronal pathways interact transsynaptically it is conceivable that catecholamines mediate stress by modifying the transmitter function. In preliminary experiments no differences were found between the values of acetylcholine turnover (measured in 6 different brain areas) in freely moving animals compared to those of rats which were immobilized with standard techniques during infusion of stable isotopes. The effect of cold exposure stress was studied by exposing rats one per cage with wet fur at 4° for various time periods. The turnover rate of acetylcholine was measured in 6 different brain areas (frontal cortex, parietal cortex, caudate nucleus, thalamus, hippocampus, and hypothalamus) after 1,4 and 24 hours of cold exposure. Exposure to cold selectively decreased the turnover rate of acetylcholine in the frontal cortex 35-40%. In other areas the turnover rates were unchanged.

Since dopamine synthesis in mesolimbic areas has been reported to be selectively increased by stress we are presently studying the possible interrelationships between the cholinergic and the dopaminergic systems of animals exposed to cold.

Publications:

Malthe-Sørenssen, D., Wood, P. L., Cheney, D. L., and Costa, E.: Modulation of the turnover rate of acetylcholine in rat brain by intraventricular injections of thyrotropin-releasing hormone, somatostatin, neurotensin and angiotensin II. J. Neurochem. 31: 685-691, 1978.

Malthe-Sørenssen, D., Cheney, D. L., and Costa, E.: Modulation of acetylcholine metabolism in the hippocampal cholinergic pathway by intraseptally injected substance P. J. Pharmacol. Exp. Ther. 206: 21-28, 1978.

Wood, P. L., Cheney, D. L., and Costa, E.: Modulation of the turnover rate of hippocampal acetylcholine by neuropeptides: Possible site of action of α-melanocyte-stimulating hormone, adrenocorticotrophic hormone and somatostatin. J. Pharmacol. Exp. Ther. 209: 97-103, 1979.

Wood, P. L., and Cheney, D. L.: The effects of muscarinic receptor blockers on the turnover rate of acetylcholine in various regions of the rat brain. Can. J. Physiol. Pharmacol. 57: 404-411, 1979.

Wood, P. L., Cheney, D. L., and Costa, E.: An investigation of whether septal GABAergic interneurons are involved in the reduction in the turnover rate of acetylcholine elicited by substance P and β-endorphin in hippocampus. Neuroscience, in press.

Wood, P. L., Cheney, D. L., and Costa, E.: A prolactin action of acetylcholine metabolism in striatum, hippocampus and thalamus. J. Neurochem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01510-04 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of cannabinoids on cholinergic and GABAergic dynamics in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Revuelta	Visiting Associate	SMRP	NIMH
Other:	P. L. Wood	Guest Worker	SMRP	NIMH
	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Δ^9-Tetrahydrocannabinol (Δ^9-THC, the active principle of marijuana, a psychoactive drug of abuse) given i.v., reduced the turnover rate of acetylcholine in hippocampus without affecting the hippocampal acetylcholine content whereas large doses of Cannabidiol, a non-psychoactive analogue of Δ^9-THC, failed to change hippocampal turnover rate of acetylcholine. (-)Δ^8-THC and nabilone which are psychoactive reduced the turnover rate of acetycholine in hippocampus while (+)Δ^8-THC which is almost devoid of psychoactive properties caused only a small reduction in the hippocampal acetylcholine turnover given in doses 20 times greater than those of (-)Δ^8-THC. Muscimol, a GABA agonist injected intraseptally, reduced the turnover rate of acetylcholine in the hippocampus. Furthermore, the inhibition by Δ^9-THC and muscimol on hippocampal turnover rate of acetylcholine could be reversed by bicuculline, a GABA antagonist, injected intraseptally.

Finally, Δ^9-THC given intravenously increased the turnover rate of GABA in septum. These results suggest that the presence of inhibitory GABAergic interneurons in septum are necessary for Δ^9-THC to specifically reduce the hippocampal turnover rate of acetylcholine.

Project Description:

The objective of this research project was to determine the effects of Δ^9-THC (the major psychoactive compound of Cannabins sativa) and related compounds on the dynamics of the cholinergic and γ-aminobutyric acid (GABA)ergic neurons in rat brain.

The techniques and methods used to measure the turnover rates of acetylcholine and γ-aminobutyric acid were described in Project No. Z01 MH 01509-08 SMRP, 1976 and in Project No. Z01 MH 01508-01 SMRP, 1976. Groups of 8 male Sprague-Dawley rats each were injected with .2,1.5,5.0 and 10 mg/kg; i.v. of Δ^9-THC or with doses of $(-)\Delta^8$-THC or $(+)\Delta^8$-THC or nabilone in doses ranging from 0.025 to 1 mg/kg; i.v. Cannabidiol was given in higher doses (5 and 10 mg/kg; i.v.). In some animals acute lesions of hippocampal cholinergic neurons were obtained by cutting the fimbria.

We found that Δ^9-THC reduced the turnover rate of acetylcholine in hippocampus in a dose related manner. Much higher doses of Δ^9-THC were required to reduce the turnover rate of acetylcholine in striatum whereas, that of parietal cortex remained unaffected. Cannabidiol, a non-psychoactive component of Cannabis sativa, did not affect the acetylcholine turnover hippocampus or of parietal cortex and only when given in high doses did it reduce the turnover rate of acetylcholine in striatum. It is tempting to speculate that the reduction in striatal acetylcholine may not be related to the psychoactive action whereas, that of hippocampus may be related to the psychoactive action.

We administered the synthetic the analogues of Δ^9-THC, $(-)\Delta^8$-THC and nabilone. We observed that both drugs reduced the turnover rate of acetylcholine in hippocampus in a dose related manner. Another analogue $(+)\Delta^8$-THC at a dose 20 times higher than that of $(-)\Delta^8$-THC caused a small reduction in the turnover rate of acetylcholine which may be due to the contamination of the starting material by the minus isomer.

To determine whether the reduction in the hippocampal turnover of acetylcholine caused by Δ^9-THC was due to a direct action on hippocampal receptor or to an indirect action through receptors located on the cell bodies of the cholinergic septal-hippocampal pathway which are located in the septum, we made an acute lesion of the pathway by cutting the fimbria. This lesion separated the cholinergic cell bodies in the septum from the nerve terminals in hippocampus and reduced the turnover rate of acetylcholine in hippocampus. There was no further reduction in the hippocampal turnover rate of acetylcholine in animals in which the fimbria had been cut. This suggests that Δ^9-THC may be acting on hippocampal turnover rate of acetylcholine via receptors located on septal cholinergic cell bodies or on neurons impinging upon these neurons.

Both Δ^9-THC and muscimol, a potent GABA agonist, reduced the hippocampal turnover rate of acetylcholine. Bicuculline, a GABA antagonist, alone had no effect on the turnover rate of acetylcholine. However, when bicuculline was given intraseptally before Δ^9-THC or muscimol, it reversed the action of both these drugs on turnover rate of acetylcholine. These results suggest that Δ^9-THC is acting through GABA interneurons in the septum.

We have previously reported (Z01 MH 01503-04, 1978) that both β-endorphin and dopamine reduce the turnover rate of acetylcholine in hippocampus by acting through GABAergic interneurons in the septum. In order to know whether or not Δ^9-THC is acting through the β-endorphinergic system we injected naltrexone intraperitoneally to block the β-endorphinergic receptors. Naltrexone did not change the turnover rate of acetylcholine in hippocampus nor did it reverse the turnover rate of acetylcholine produced by Δ^9-THC.

To destroy the dopamine terminals innervating the septum 6-hydroxydopamine was injected into area A_{10} through a chronically implanted cannula. We measured the levels of dopamine and norepinephrine in the septum. 6-Hydroxydopamine decreased the dopamine level in the septum by 75%. Concomittantly the turnover rate of acetylcholine in hippocampus was increased significantly. Δ^9-THC, when given after 6-hydroxydopamine, showed a marked reduction in the turnover rate of acetylcholine in hippocampus. These experiments would suggest that the action of Δ^9-THC on the cholinergic septal-hippocampal pathway does not involve endorphinergic or dopaminergic neurons. However, these experiments do not exclude the possibility that Δ^9-THC activates a receptor for an unknown excitatory transmitter released by axon terminals afferent to septal GABAergic neurons.

Since Δ^9-THC acts through a GABAergic interneuron to reduce the turnover rate of acetylcholine this suggests that GABA turnover, in this nucleus, may be increased as a result of Δ^9-THC administration. We measured the turnover rate of GABA in septum and found that 5 mg/kg of Δ^9-THC i.v. can increase the turnover rate of GABA in the septum, selectively. Only at higher doses changes in turnover rate of GABA are seen also in other brain areas.

From the biomedical point of view this investigation can help us to better understand the mechanism whereby Δ^9-THC and other related analogues of the widely used Cannabis sativa act to modify mood and perception. Furthermore, this research can suggest that specifical septal receptors which are activated by the cannabinoids.

Publications:

Revuelta, A. V., Moroni, F., Cheney, D. L., and Costa, E.: Effect of cannabinoids on the turnover rate of acetylcholine in rat hippocampus, striatum and cortex. Naunyn-Schmiedebergs Arch. Pharmacol. 304: 107-110, 1978.

Revuelta, A. V., Cheney, D. L., Wood, P. L., and Costa, E.: GABAergic mediation in the inhibition hippocampal acetylcholine turnover rate elicited by Δ^9-tetrahydrocannibinol. Neuropharmacology 18: 525-530, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01511-06 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neuronal monoamines: A study of their integration and regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	F. Vargas	Guest Worker	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The integrated function of dopaminergic, GABAergic and substance P neurons was investigated in striatum and N. accumbens after short- and long-term treatment with antischizophrenic drugs. The data suggest that GABA and substance P terminals in SN may be involved in mediating the effect of antischizophrenic drugs on nigro-striatal DA system. Long-term treatment with classical (cataleptogenic) antischizophrenic drugs produces tolerance to the activation of the DA striatal system. Tolerance is not observed after long-term treatment with atypical (non-cataleptogenic) antischizophrenic drugs. In striatum the supersensitivity of DA receptors produced by these drugs is associated with an increase in calcium binding protein (calmodulin). These data suggest that the DA neurons adapt to the effects elicited by antischizophrenic drugs when supersensitivity of DA receptors develops.

PHS-6040
(Rev. 10-76)

Project Description:

Though the catecholamine (noradrenaline and dopamine) hypothesis of affective disorders has received support during the past decade, it is today evident that catecholamines are not the only transmitter system that may be involved in the pathogenesis of affective disorders, in fact, not all aspects of the pathophysiology of various forms of affective disorders can be explained by simple alterations of catecholamine metabolism. Clearly, many other concomitant biochemical, physiological, and psychological factors must be involved.

During the last years, many studies have demonstrated the complex anatomical, physiological and biochemical interactions among monoaminergic and other neuronal systems. In this project we intend to study the consequences of short- and long-term treatment with neuroleptics endowed with antischizophrenic activity on the dopaminergic and other synapses that interact with dopamine neurons.

1) The antischizophrenics which elicit a high incidence of extrapyramidal side effects (termed "classical") preferentially increase dopamine turnover in striatum. The antischizophrenics which cause a low incidence of extrapyramidal side effects (termed "atypical") preferentially increase the turnover of DA in N. accumbens.

2) Discrete lesions of neuronal pathways descending from striatum to substantia nigra (SN) abolish the ability of antischizophrenic drugs to activate striatal tyrosine hydroxylase (TH). These lesions resulted in a 60-90% decrease of GABA and substance P content in SN without changing the DA content of SN or striatum. Application of GABA-mimetic compounds such as muscimol or diazepam, either systemically or directly into SN, block the activation of striatal TH elicited by classical antischizophrenics. On the other hand, systemic administration of GABA antagonists such as picrotoxin or GABA synthesis blockers such as isoniazid potentiate the action of haloperidol on striatal TH. These data suggest that GABA terminals and possibly also substance P terminals in SN, mediate the activation of striatal TH elicited by classical antischizophrenics.

3) Long-term treatment with "classical" (cataleptogenic) and "atypical" (non-cataleptogenic) neuroleptics produces differential effects on the TH activity of locus coeruleus, C. striatum and N. accumbens. The activity of TH (Vmax) in locus coeruleus but not in striatum or nucleus accumbens is increased in rats treated for a long period (10 to 90 days) with 0.5 mg/kg/day of haloperidol.

Upon chronic haloperidol treatment and subsequent withdrawal for 3, 15 or 30 days, challenging doses of haloperidol or other "classical" neuroleptic drugs (pimozide, chlorpromazine, methiotepine) fail to increase the affinity of striatal TH for DMPH4. Such tolerance to haloperidol was also present in N. accumbens however in this tissue the tolerance disappears 7 days after haloperidol withdrawal. After chronic haloperidol there is not cross-tolerance with the clozapine or sulpiride-induced increase of striatal TH affinity for

$DMPH_4$. Tolerance to long-term treatment with classical neuroleptics was not prevented by long-term treatment with Li^{++}. The rat treated chronically with clozapine and sulpiride, there was no tolerance to the effects on TH by challenging doses of clozapine or haloperidol.

4) Long term treatment with classical neuroleptics in addition to tolerance to the increase of striatal TH elicited by these drugs, generates supersensitivity to the behavioral effects of apomorphine. The supersensitive response of DA receptors is associated with an increase in the striatal content of Ca^{++} binding protein (calmodulin).

To enhance our level of understanding of the role of calmodulin in the DA receptor function we have measured calmodulin content in striatum and nucleus accumbens of rats after transsection of the striato-nigral pathway. The calmodulin content increases in striatum but not in N. accumbens. In addition we have measured the calmodulin content in striatum and cortex of 9 control subjectes and 11 schizophrenic patients but no differences were observed in the calmodulin content between the two groups.

These results indicate that long-term treatment with classical antischizophrenic produces complex adaptive changes in the catecholamine system. The changes elicited by repated injections of antischizophrenic durgs favor the hypothesis that mesolimbic DA is a site involved in the control of affective disfunction by antipsychotics.

In addition, the rapid and long lasting tolerance to the stimulation of TH observed in striatum of rats repeatedly treated with antischophrenic drugs relates to the process of supersensitivity of striatal DA receptors. This supersensitivity may be considered a biochemical determinant of the tardive dyskinesia which is observed in man after discontinuation of the treatment or the reduction of the dosage of classical antischizophrenic drugs.

Several reasons account for the differential development of tolerance and supersensitivity to the stimulation of TH in striatum and N. accumbens: among others a different synaptic wiring in the two nuclei could play a role. For instance, in N. accumbens but not in striatum, the GABAergic neurons are innervated by dopaminergic neurons. We, therefore, plan to determine more precisely the role of the striato-nigral pathway and striatal and accumbens interneuronal connections in the regulation of DA neuronal function. In particular, we want to study the respective influence of nigra and striatal substance P, GABA, and opiate peptides or opiate agonists and antagonists in the regulation of DA activity. In addition, we plan to study the role of cortico-striatal glutamatergic projections. According to general clinical experience the full therapeutic response to a neuroleptic agent may require several weeks and may last for a considerable time after cessation of therapy. There are reasons to believe that the primary actions of neuroleptics, as observed in short-term experiments, is only the first response to induce a series of secondary effects, the nature of which has not yet been fully clarified. We thus, propose to extend our studies to the different neuronal system associated with the DA system and to long-term teratment with "classical" and "atypical" antipsychotics.

Publications:

Luchelli, A., Guidotti, A., and Costa, E.: Striatal content of Ca^{++} dependent regulator protein and dopaminergic receptor function. Brain Res. 115: 130-135, 1978.

Gale, K., Costa, E., Hong, J., Toffano, G., and Guidotti, A.: Evidence for a role of nigral GABA and substance P in the haloperidol induced activation of striatal tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 206: 20-37, 1978.

Guidotti, A., Gale, K., Toffano, G., and Vargas, F. M.: Tolerance to tyrosine hydroxylase activation in N. accumbens and C. striatum after repeated injections of "classical" and "atypical" neuroleptics. Life Sci. 23: 501-506, 1978.

Guidotti, A.: Regulation of tyrosine hydroxylase after chronic treatment with classical and atypical antischizphrenic drugs. In Cattabeni, F., Racagni, R. and Spano, P. F. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, in press.

Vargas, F.: Calmodulin content in human brain. In preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01512-06 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Transmitter interactions in the regulation of pituitary function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Grandison	Staff Fellow	SMRP	NIMH
Other:	M. Baraldi	Visiting Associate	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

None

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of GABA, catecholamine and endorphins in the response of hypothalamus to different pyschoactive drugs was studied by monitoring pituitary hormone release. Endogenous opiate stimulate PRL and block FSH release by activation of hypothalamic opiate receptors. These opiate receptors may be located on cells containing prolactin releasing factor since median eminence DA neurons or serotoninergic neurons apparently do not mediate the effects of morphine. Stimulation of hypothalamic or brain GABA receptors with muscimol fails to block morphine or haloperidol-induced PRL release. In contrast stimulation of GABA receptors in anterior pituitary prevents the morphine or haloperidol-induced PRL release.

PHS-6040
(Rev. 10-76)

Project Description:

Hypothalamic neuronal mechanisms transsynaptically regulate behavior and secretion of polypeptide releasing factors (RF's). The RF's, in turn, regulate pituitary function by stimulating adenylate cyclase in the anterior pituitary. In order to define the type of neurotransmitters involved in the release of pituitary hormones, the effects of neuroactive drugs were monitored by the measurement of pituitary hormone release.

1) Effect of opiate receptor agonists and antagonists

Injection of β-endorphin into the basomedial hypothalamus induced an increase of PRL release. Naltrexone was able to block this response. Specificity was further demonstrated since a 10 fold higher dose of α-endorphin (inactive peptide) was ineffective. When injected into the hypothalamus naltrexone itself produced a suppression of PRL release. After deafferentation morphine still stimulated and naltrexone still inhibited PRL release. In contrast to its activity in vivo, β-endorphin ($2x10^{-8}$M; $2x10^{-7}$M) or naltrexone (10^{-4}M) had no effect on PRL release from the pituitary in vitro. In addition, β-endorphin was unable to alter the inhibitory action of $5x10^{-7}$M apomorphine on in vitro pituitary halves.

Next, we attempted to determine the events after stimulation of hypothalamic opiate receptors that induce PRL release. When the DA tonus was potentiated by giving pargyline (a monoaminoxidase inhibitor) PRL release following submaximal doses of morphine or β-endorphin was not altered. In contrast, when the DA activity was reduced by administration of reserpine or haloperidol, pargyline significantly reduced the increase release of PRL. In addition, the action of morphine or β-endorphin but not that of reserpine or haloperidol was blocked by naltrexone. Pretreatment of the animals with PCPA in doses that significantly inhibit tryptophan hydroxylase activity or intrahypothalamic injection of muscimol (a GABA mimetic) did not alter morphine induced PRL release.

2) Effect of GABA receptor agonists

The rise in serum prolactin (PRL) concentration after haloperidol (0.05 to 0.5 mg/kg i.v.) or morphine (2 to 16 mg/kg s.c.) in male rats and during estrogen treatment (1 mg/day/15 days) in ovariectomized rats was reduced by systemic injection of GABA receptor agonist muscimol (1 mg/kg i.v.). However, intraventricular or intrahypothalamic injection of muscimol (100-400 ng) was ineffective in blocking PRL release induced by these compounds.

Since the distribution and concentration of muscimol in brain after central administration was similar to that following systemic injection, these observations demonstrate that stimulation of central GABA receptors was not involved in the regulation of PRL release during these processes. Rather the high levels of muscimol in the anterior pituitary after systemic injection and its absence after central administration suggested that blockade of PRL release by muscimol might result from a stimulation of GABA receptor located in the pituitary. Indeed, ^{3}H-muscimol and ^{3}H-GABA receptor recognition sites with characteristics similar to that of brain were present in rat anterior pituitary. In addition, muscimol (10^{-8} to 10^{-6}M) reduced the spontaneous release of PRL from incubated anterior pituitary halves in vitro. This effect was reversed by the GABA receptor antagonist, bicuculline, but not by the dopamine antagonist, sulpiride. These results provide evidence that GABA receptor stimulation in the anterior pituitary can act to regulate the release of PRL. These data suggest that GABA mimetic drugs which fail to enter the brain may represent a relevant approach to control the PRL-releasing action of neuroleptics without altering their central effects.

These data suggest that catecholamines, GABA and endorphins are all involved in regulation of anterior pituitary functions. From these studies it appears that different neurotransmitter systems are involved in the release of specific anterior pituitary hormones. For example, PRL release is under hypothalamic DA and endorphins control but not under a direct hypothalamic GABA control. GABA might control PRL release acting directly at pituitary level independently from catecholamines. Further efforts are now required to extend these studies to the numerous hypothalamic peptides neurotransmitter substances (neurotensin, somatostatin, substance P, bombesin) which have been shown to influence hormone release.

These studies are relevent to understanding total brain function because the hypothalamic neuronal peptides investigated here are now known to be widely distributed throughout the brain. The hormone release-neurotransmitter function relationship in the hypothalamus pituitary axis may be used as a tool to learn the action of different psychoactive drugs on neuropeptidergic transmission.

Publications:

Baraldi, M., Grandison, L., and Guidotti, A.: Distribution and metabolism of muscimol in the brain and other tissue of rat. Neuropharmacology 18: 57-62, 1979.

Guidotti, A., and Grandison, L.: Participation of endorphins in the regulation of pituitary function. In Usdin, E., Bunney, W. E. and Kline, N. J. (Eds.): Endorphins in Mental Health Research. pp. 416-422, 1979.

Grandison, L., and Guidotti, A.: γ-Aminobutyric acid function in the rat anterior pituitary: Evidence for control of prolactin release. Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01514-07 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Trans-synaptic control of protein synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Kumakura	Visiting Fellow	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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0.3	0.3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the primary culture of chromaffin cells from cow adrenal medulla TH induction is preceded by an activation of cytosol cAPK and by an increase in nuclear protein phosphorylation. Both the induction of TH and the increase of nuclear phosphorylation require that the assembly of microtubular proteins is functional. Antimicrotubular drugs such as colchicine and vinblastine (10^{-9}M) can block the TH induction elicited by 8-Br cAMP, when the drugs are added less than 15 hrs after 8-Br cAMP. Since colchicine, added within cAMP also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the intracellular translocation and nuclear uptake of catalytic subunits of cAPK activated by the addition of 8-Br cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediation of the acceleration of mRNA synthesis and the successive increase in TH synthesis elicited by 8-Br cAMP.

Project Description:

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffin cells of adrenal medulla.

In previous studies we reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the genetic code expression includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the transsynaptic induction of tyrosine-3-monooxygenase (TH) because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP due to the activation of cAPK acts as a long range messenger for the transsynaptic expression of the genetic code.

An injection of nicotinic receptor antagonist or the denervation of adrenal medulla reduces and shortens the cAMP elevation caused by cold exposure and it prevents the cAPK activation, it inhibits the nuclear translocation of cAPK catalytic subunits and also it blocks the induction of TH. Though these experiments demonstrate a temporal sucession between the translocation of the catalytic subunits of cAPK to the nucleus, the increase in nuclear protein phosphorylation and the stimulation of gene activity leading to an increase in TH synthesis, they fail to elucidate: 1) the specific regulatory mechanism involved in the nuclear uptake of catalytic subunits of cAPK; 2) the precise role in nuclear function of the subunits of cAPK that translocate to the nucleus; 3) the mechanisms underlying gene transcription specificity; and 4) the necessity of nuclear translocation of cAPK catalytic subunits in eliciting the transsynaptic induction of TH.

In vivo experiments with rat adrenal medulla are not the most suitable to study transsynaptic regulation of gene expression at molecular level because rat adrenal medullae provide only a limited amount of purified nuclear material which does not allow a rigorous monitoring of the fine biochemical events occurring in the nuclear chromatine.

We have then decided to use as an in vitro system primary culture of cow adrenal medulla cells. Thse cells were used as a model to evaluate the ability of 8-Br cyclic AMP (8-Br cAMP) to induce TH and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines and related enzyme activities for about four weeks.

Exposure of the cells for 5 hrs to 8-Br cAMP, produces 48 hrs later a dose related longlasting increase in the TH activity; 8-Br cGMP fails to modify TH. The increase in TH activity caused by 8-Br cAMP is due to an increase of the Vmax and is preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphorylation begins 8 to 12 hrs after 8-Br cAMP application. The delayed increase in TH activity induced by 8-Br cAMP is blocked by actinomycin D, cycloheximide, colchicine and vinblastine. This reduction of the TH induction elicited by colchicine and vinblastine (10^{-9}M) is observed only when these inhibitors of the microtubular protein polymerization were added 4 to 12 hrs after the addition of 8-Br cAMP which is the inducing stimulus. The addition of colchicine 15 hrs after 8-Br cAMP fails to inhibit TH induction. This blockade of TH induction is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH was also produced by cholera toxin. These results suggest that the increase of TH elicited by 8-Br cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network. In future studies we plan to study how the increase in nuclear phosphorylation regulate the expression of the genetic code during the induction of TH.

Since in the adrenal medulla, the availability of catecholamines and the adrenergic function are regulated by TH and since adrenergic mechanisms have been implicated in the etiology of affective disorders, an understanding of the molecular nature of the regulation of biosynthesis of catecholamines may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition the translocation of cAPK subunits may operate as a basic mechanisms in memory and/or learning.

Publications:

Kurosawa, A., Guidotti, A., and Costa, E.: Nuclear translocation of cyclic AMP-dependent protein kinase subunits during the transsynaptic activation of gene expression in rat adrenal medulla. Mol. Pharmacol. 15: 115-130, 1979.

Kumakura, K., Guidotti, A., and Costa, E.: Role of protein kinase translocation in the induction of tyrosine 3-monooxygenase (TH) in adrenal chromaffin cell cultures. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, in press.

Kumakura, K., Guidotti, A., and Costa, E.: Primary cultures of chromaffin cells: Molecular mechanisms for the induction of tyrosine hydroxylase mediated by 8-Br cyclic AMP. Mol. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01515-06 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Short and long term biochemical changes in cerebellum after treatment with psychoactive drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Bandle	Visiting Associate	SMRP	NIMH
Other:	G. Toffano	Guest Worker	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemically, the cerebellum is characterized by a large concentration of GABA, cAMP, cGMP-dependent protein kinase and a heat-stable protein which inhibits the cGMP-dependent protein kinase. Drug-induced changes in GABA receptor function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that diazepam and muscimol (an indirect and a direct GABA receptor agonist), modulate cerebellar phosphorylation by altering the relationship between cGMP-dependent protein kinase and the content of the cerebellar inhibitor of protein kinase. They could act on the content of the protein kinase inhibitor by decreasing the cerebellar cGMP content without modifying that of cAMP. Since very recently we have established that the endogenous inhibitor of protein kinase is identical to the inhibitor of GABA receptor binding (GABA-modulin), the cerebellum became the organ of choice where to study the relationship between GABAergic function, phosphorylation of selective membrane proteins and GABA-modulin content.

PHS-6040
(Rev. 10-76)

Project Description:

The cerebellum is the brain region where the synaptic interactions among various neurons has been determined with greater detail and precision. The project is designed to relate changes of cAMP, cGMP content and protein kinase activities with the release of transmitters in the cerebellar cortex and deep cerebellar nuclei. Such studies are possible in cerebellum because:

1. The climbing fibers and the mossy fibers are the two major excitatory inputs to the Purkinje cells.

2. The function of Purkinje cells is regulated by inhibitory (GABAergic) interneurons.

3. The Purkinje cells are the sole output from the cerebellum and release GABA on the deep cerebellar neurons.

In previous reports we have shown that pharmacological manipulations of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebellar GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the firing rate of Purkinje cells and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the firing rate of Purkinje cells and the cerebellar cGMP content are increased.

These results suggest that two excitatory transmitters released by the parallel or climbing fibers and GABA released by cerebellar interneurons are independently involved in changing cGMP content on the molecular layer of cerebellum.

As we have previously reported (Szmigielski, A., Guidotti, A., Costa, E. J. Biol. Chem. 252: 3848-3853, 1977) the rat cerebellum contains a significant amount of cGMP-dependent protein kinase and a large concentration of protein kinase inhibitors. These inhibitors are thermostable proteins which can be separated by gel chromatography into 2 molecular forms: the Type I and Type II inhibitor of protein kinase. The Type I inhibitor blocks the rat cerebellar cGMP-dependent protein kinase activity while the Type II inhibitor blocks the rat cerebellar cGMP-dependent protein kinase and the cyclic nucleotide independent protein kinase. The activity of the Type II inhibitor increased or decreased in opposite direction to changes of cerebellar cGMP content generated by injection of harmaline or diazepam. No changes of Type I inhibition were observed under thse conditions.

The drug-induced shift of Type II inhibitor of protein kinase was not mediated by changes in protein synthesis because it persisted after pretreatment with cycloheximide. These results are compatible with the hypothesis that cGMP modulates phosphorylation in cerebellum by changing the relationship between cGMP-dependent protein kinase and Type II inhibitor content. Experiments were then directed to elucidate the possible message mediated by the increase of cGMP in the cerebellum. To achieve this goal our attention has been focused on cGMP dependent protein kinase and on the thermostable protein inhibitors of protein kinase dependent phosphorylation. We have developed a simple method to assay cGMP-dependent protein kinase which allows the simultaneous processing of a large number of samples. Using this method we have established that cerebellar cGMP-dependent protein kinase is very low at birth and increases with age in coincidence with several ontogenetic events in cerebellum. One of the events that more closely relate with the increase of cGMP dependent protein kinase is the formation of a permanent synaptic contact between climbing fibers and Purkinje cells. In addition using mutant mice (nervous, weaver, staggerer) and rats treated with 3-acetylpryidine we further established a fundamental role of intact climbing fiber-dendritic synaptic contacts for the maintanence of normal cGMP-dependent protein kinase activity in cerebellum.

Considering the important role of the cerebellum in the regulation of the sensory motor cortex, function, and the large abundance of inhibitory (GABA, taurine) or excitatory transmitters (aspartic and glutamic acid) it is conceivable that studies on the modification of cerebellar cGMP system may become an important tool to study the action of anti-psychotic tranquilizer and antidepressant drugs.

Our proposed course is to define the molecular nature of the messages mediated by the increase of cGMP. We have now established that rat cerebellum contains (1) a large number of cGMP-dependent protein kinase and (2) high concentrations of a thermostable small molecular protein which inhibits cAMP dependent and cGMP-dependent protein kinase. In addition, we have established that the protein inhibitor of protein kinase is identical to the modulator of GABA receptor binding.

We intend to study whether the transmitter-mediated changes of cerebellar cGMP-dependent protein kinase are paralleled by changes of GABA receptor binding and by changes in the content of the endogenous protein inhibitor of the protein kinase and of GABA binding.

Publications:

Guidotti, A., Biggio, G., and Costa, E.: Action of diazepam, haloperidol, morphine and muscimol on the cGMP content of cerebellum. In Deniker, P., Raduco Thomas, D., and Villeneuve, A. (Eds.): Neuropsychopharmacologicum. Oxford, England, Pergamon Press, in press.

Bandle, E., and Guidotti, A.: Studies on the cell location of cyclic 3',5'-guanosine monophosphate-dependent protein kinase in cerebellum. Brain Res. 156: 412-416, 1978.

Bandle, E., and Guidotti, A.: Ontogenetic studies of cGMP-dependent protein kinase in rat cerebellum. J. Neurochem. 32: 1343-1347, 1979.

Szmigielski, A., and Guidotti, A.: Action of harmaline and diazepam on the cerebellar content of cGMP and the activities of two endogenous inhibitors of protein kinase. Neurochem. Res. 4: 189-200, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01516-06 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of minor tranquilizers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Baraldi	Visiting Associate	SMRP	NIMH
Other:	G. Toffano	Guest Worker	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH
	D. Konkel	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH
	A. Carenzi	Guest Worker	SMRP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular mechanism whereby GABAergic transmission is modified by benzodiazepines (BDZs) was studied in vivo and in vitro. In vivo BZDs like the GABA mimetic, muscimol antagonize the isoniazid-induced convulsions, and the isoniazid-induced increase of cerebellar cGMP and pituitary cAMP content. In vitro BDZs facilitate binding of GABA to its receptor sites by competing with the endogenous membrane protein (GABA-modulin) which acts as an inhibitor of high affinity Na^+-independent GABA binding to synaptic membrane preparations. GABA-modulin competes with BDZs for their specific receptor sites. GABA, benzodiazepine receptors and GABA-modulin are present in the membranes of a neuroblastoma cell line. In these cells stimulation of GABA receptor increases Cl^- influx and BDZs facilitates the action of GABA. This increase in the binding affinity of GABA for its postsynaptic site may represent the molecular mechanism by which BDZs facilitate GABAergic transmission in vivo. These observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZs receptors and also may offer a way to study the biochemical defects in anxiety states.

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines, which are the most widely prescribed drug, may exert a beneficial effect in psychiatric disturbance by a primary action on GABA mechanisms. The present project intends to elucidate the molecular mechanisms of action of benzodiazepines.

BZDs like muscimol (a direct GABA receptor agonist) and intraventricular nipecotic acid (a blocker of GABA uptake) delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from strychnine convulsions. Moroever, diazepam and muscimol had a similar potency in preventing the increase of cerebellar cGMP or of pituitary cAMP induced by isoniazid, picrotoxin, harmaline and reserpine. In the nigrostriatal system BZDs, similarly to GABA receptor agonists, block the activation of DA neurons induced by antipsychotics.

The increase in punished behavior elicited by benzodiazepines in rats operating in a conflict test situation is potentiated by intraventricular injections of muscimol. Muscimol, injected intraventricular in doses of 50 to 200 ng also produces a benzodiazepine-like action by increasing the output of punished operating behavior. These data concur with the hypothesis that the pharmacological action of BZDs may be mediated by an effect on GABA neurons.

To examine at the molecular level whether BZDs changed the binding of ^{3}H-GABA to postsynaptic receptors we measured whether BDZs changed the binding of GABA to freshly prepared synaptic plasma membranes or to membrane treated with Triton X-100 frozen and thawed repeatedly. BDZs appear to unravel a high affinity component for the binding of GABA to fresh membranes but fail to change the GABA binding to frozen and thawed synaptic plasma membranes. Similar results were obtained using clonazepam, flunitrazepam, nitrazepam and (+)-RO-11-3128 (an active BDZ) but not (-)-RO-11-3624 (an inactive isomer). In these membranes there are high affinity and low affinity GABA binding whereas in freshly prepared membranes there are only low affinity binding sites for GABA.

Freshly prepared synaptic membranes contain an acidic protein which inhibits the Na^+-independent high affinity binding of ^{3}H-GABA. This inhibitor GABA-modulin is thermostable, has a MW of about 15,000 and has been purified 500-fold. When the membranes are frozen, thawed, repeatedly washed and then treated with Triton X-100 the GABA-modulin dissociates from the membranes. Kinetic analysis suggests that the GABA-modulin acts as an allosteric modulator for the Na^+-independent high affinity GABA binding. Diazepam ($5x10^{-7}$M) prevents the inhibition by this protein of ^{3}H-GABA binding to frozen and detergent-treated membranes.

These results suggest that BDZs compete with the endogenous inhibitor for the Na^+-independent high affinity binding of GABA. It has been reported that benzodiazepines work on specific "anxiety" receptors. In recent experiments we have observed that the endogenous inhibitor of GABA binding also inhibits ^{3}H-diazepam binding. One question posed by these results is whether the interaction shown in vitro in brain synaptic membranes between GABA and benzodiazepines could be just the result of homogenization or whether the three entities (GABA, benzodiazepine receptor and GABA-modulin) were part of a supramolecular organization constituting the GABA receptor-ionophore complex at the receptive site of the post-junctional neuronal membranes. To develop a simple model where to study in vivo the interaction between GABA, benzodiazepine receptor and GABA-modulin we have used different cell lines: neuroblastoma; NB2a, gioma C6, chromaffin cells from bovine adrenal and P_{12} cell line.

A "GABA receptor unit" which includes brain-like ^{3}H-GABA, ^{3}H-benzodiazepine (diazepam, clonazepam, flunitrazepam) receptor binding sites and a thermostable endogenous protein (GABA-modulin) which inhibits both GABA and benzodiazepine binding has been demonstrated to be present in membranes prepared from NB2a neuroblastoma (NB) and C6 glioma (C6) clonal cell lines. In these cells, as in brain, diazepam (1 μM) prevents the effect of GABA-modulin and in turn GABA (0.1 mM) increases the binding of ^{3}H-diazepam. The NB, and to a lesser extent C6 cells, represent a suitable model to study the interactions and the sequence of membrane and intracellular events triggered by stimulation of benzodiazepine and GABA receptors. In fact in these cells muscimol and GABA increase the Cl^- influx and this increase is blocked by bicuculline. Diazepam which produces release of GABA-modulin from these cells also facilitate the increase of Cl^- flux elicited by GABA.

In conclusion, our data suggested that BZD receptors are functionally associated with GABA receptors. Thus, the pharmacological and therapeutic action of BDZs may be mediated by an action on GABAergic neurons.

The importance of these observations may go beyond the immediate explanation of the mode of action of BDZs and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders. In addition, these observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a simple means for studying the GABAergic pathway involved in anxiety.

Publications:

Guidotti, A.: Methods to evaluate in vivo the activity of GABA receptor agonists. In Deniker, P., Raduco-Thomas, C. and Villeneuve, A. (Eds.): Proceedings of the 10th Congress of Collegium Internationale Neuropsychopharmacologium. Oxford, England, Pergamon Press, pp. 439-446, 1978.

Toffano, G., Guidotti, A., and Costa, E.: Purification of an endogenous protein inhibitor of the high affinity binding of GABA to synaptic membranes of rat brain. Proc. Natl. Acad. Sci. USA 75: 4024-4028, 1978.

Guidotti, A., Toffano, G., Grandison, L., and Costa, E.: Second messenger responses and regulation of high affinity receptor binding to study pharmacological modifications of GABAergic transmission. In Fonnum, E. (Ed.): Amino Acids as Chemical Transmitters. New York, Plenum, Publ., pp. 517-530, 1978.

Guidotti, A., Biggio, G., and Costa, E.: Action of diazepam, haloperidol, morphine and muscimol on the cGMP content of cerebellum. In Deniker, P., Raduco-Thomas, C. and Villeneuve, A. (Eds.): Neuropharmacology. Oxford, England, Pergamon Press, pp. 917-924, 1978.

Guidotti, A., Toffano, G., Baraldi, M., Schwartz, J. P., and Costa, E.: A molecular mechanisms for the facilitation of GABA receptor function by benzodiazepines. In Krogsgaard-Larsen, Scheel-Krueger, J. and Kofon, H. (Eds.): GABA: Pharmacochemical, Biochemical and Pharmacological Aspects. Munksgaard, Copenhagen, pp. 406-415, 1979.

Costa, E., and Guidotti, A.: Molecular mechanism in the receptor action of benzodiazepines. In Okun, G. R. and Cho, A. K. (Eds.): Annual Review of Pharmacology and Toxicology. Vol. 19, Palo Alto, Calif., An. Rev. Inc., pp. 531-545, 1979.

Guidotti, A., Baraldi, M., Schwartz, J. P., Toffano, G., and Costa, E.: A molecular mechanism for the action of benzodiazepines on GABAergic transmission. In Simon, P. (Ed.): Advances in Pharmacology and Therapeutics, Vol. 2. Neurotransmitters. Oxford, England, Pergamon Press, pp. 33-41, 1978.

Costa, E., Guidotti, A., and Toffano, G.: Molecular mechanisms mediating the action of diazepam on GABA receptors. Brit. J. Psychiat. 133: 239-248, 1978.

Guidotti, A., Toffano, A., and Costa, E.: An endogenous protein inhibits Na^+ independent GABA binding and functions as a ligand for benzodiazepine receptors in brain. Nature 275: 553-555, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01517-04 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of a high power microwave oven

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology and Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Focussed microwave irradiation does not impair microdissection of rat brain nuclei, spinal cord and other tissues and allows the assay of the concentrations of chemicals which undergo rapid metabolism during postmortem processes. New microwave devices to inactivate enzymes in brain and other tissues of large animals and in free moving animals are needed. Such equipment would offer a unique possibility of measuring the effect of drugs on the steady state and turnover rate of neurotransmitters of metabolites during various stages of sleep.

PHS-6040
(Rev. 10-76)

Project Description:

We have attempted to increase the value of microwave radiation in studying putative transmitters in spinal cord and brain of rats by increasing the intensity of the beam. The new microwave source allows a rapid (2-3 sec.) inactivation of the enzymes controlling cAMP and cGMP metabolism in spinal cord and in organs such as kidney, spleen, heart, etc., without handling the animals. A high power microwave generator that would allow us to focus the microwaves on the skull of an unrestrained animal should allow important progress in the studies of different neurotransmitters and metabolites in spinal cord or brain during various stages of sleep.

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01518-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The study of mammalian GABA-ergic mechanisms with gas chromatography-mass spectrometry and stable isotopes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Peralta	Guest Worker	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of dopaminergic and glutamatergic inputs on the activity of GABA neurons in various areas of the brain has been studied. Electrical stimulation of the substantia nigra and A10 area was used to obtain an enhanced dopaminergic activity in N. caudatus and N. accumbens. Ablation of the cortex was employed to suppress glutamatergic input into N. caudatus. The activity of GABAergic neurons was assessed by measuring the turnover rate of GABA using a gas chromatographic-mass spectrometric technique. In one experiment, a similar technique was employed to study the activity of cholinergic neurons. Our results show that in N. accumbens, but not in N. caudatus, dopamine directly inhibits GABA neurons. In N. caudatus glutamatergic fibers originating in the cortex stimulate the activity of GABA and acetylcholine neurons.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective has been to study the modulation of GABA interneurons in various nuclei of rat brain by dopaminergic and glutamatergic inputs to these nuclei.

Previous work has suggested that cholinergic interneurons are under direct inhibitory influence by dopamine in both N. caudatus and N. accumbens whereas GABAergic interneurons are also directly inhibited by dopamine in N. accumbens, but not in N. caudatus. In order to further confirm this hypothesis, we have estimated the turnover rate of GABA in both structures following electrical stimulation of the substantia nigra and A_{10} area, which are known to contribute most of the dopaminergic input to N. caudatus and N. accumbens, respectively. The turnover rate of GABA was measured by a sensitive gas chromatography-mass fragmentography technique involving infusion of ^{13}C-glucose through the tail vein of conscious immobilized animals and determining the relative isotopic enrichment attained by the pools of GABA and its immediate precursor, glutamic acid.

Electrical stimulation of the substantia nigra or A_{10} area produces an increase in homovanillic acid content of the homolateral N. caudatus and N. accumbens, respectively. No consistent changes in caudatal turnover rate of GABA could be detected in connection with this enhanced dopaminergic activity. In N. accumbens however, dopamine activation was associated with a significant decrease of the turnover rate of GABA in the stimulation side with respect to the control side.

Glutamatergic input to caudatal neurons is mediated essentially through a cortico-striatal pathway. In order to determine whether this pathway modulates striatal GABAergic function, we measured the turnover rate of GABA in striatum after chronic decortication. We found that two weeks after decortication the uptake and content of striatal glutamate are significantly reduced while GABA uptake remains unchanged. The turnover rate of GABA, however, was markedly reduced after decortication. We extended these studies to cholinergic neurons and found that glutamatergic deafferention of the N. caudatus also decreases the turnover rate of acetylcholine in this nucleus.

The results summarized above suggest that: a) GABAergic interneurons are directly inhibited by dopamine in the N. accumbens, but not in the N. caudatus. b) In this latter nucleus, glutamatergic fibers originating in the cortex represent a strong positive input to GABAergic interneurons. c) Similarly, striatal cholinergic interneurons are under stimulatory control of the glutamatergic cortico-striatal pathway.

The study of these interactions among different neuronal systems in the brain is essential to attain an elucidation of the mechanism of action of psychotropic drugs. Previous work from our laboratory has indicated that GABAergic might be involved in the therapeutic action and cholinergic mechanisms mediate the undesirable side effects of antipsychotic drugs. Because these drugs are known to exert a blockade of dopamine receptors, most of the work attempting to disclose their mechanism of action has focussed on dopaminergic neurotransmission alone. Demonstration on the intricate relationship between these and other types of chemical transmission in the central nervous system will contribute to our better understanding of how the diverse systems of the brain respond to antipsychotic therapy, thus providing us with the background knowledge necessary to develop more effective therapeutic approaches.

Publications:

Marco, E., Mao, C. C., Revuelta, A., Peralta, E., and Costa, E.: Turnover rates of γ-aminobutyric acid in substantia nigra, N. caudatus, globus pallidus and N. accumbens of rats injected with cataleptogenic and non-cataleptogenic antipsychotics. Neuropharmacology 17: 589-596, 1978.

Green, A. R., Peralta, E., Hong, J. S., Mao, C. C., Atterwill, C. K., and Costa, E.: Alterations in GABA metabolism and met-enkephalin content in rat brain following repeated electroconvulsive shocks. J. Neurochem. 31: 607-611, 1978.

Mao, C. C., Peralta, E., Moroni, E., and Costa, E.: The turnover rate of γ-aminobutyric acid in the substantia nigra following electrical stimulation or lesioning of the strio-nigral pathways. Brain Res. 155: 145-152, 1978.

Moroni, F., Peralta, E., and Costa, E.: Turnover rate of GABA in striatal structures: Regulation and pharmacological implications. Alfred Benzon Sympl., 12, Munksgaard 1978.

Wood, P. L., Moroni, F., Cheney, D. L., and Costa, E.: Cortical lesions modulate turnover rates of acetylcholine and γ-aminobutyric acid. Neurosci. Lett., in press.

Peralta, E., and Costa, E.: Effect of electrical stimulation of the substantia nigra and A10 area on the turnover rate of GABA in N. caudatus and N. accumbens. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01519-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The biochemistry of cholera toxin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Trepel	Biol Lab Tech	SMRP	NIMH
	L. M. Neckers	Staff Fellow	SMRC	NIMH
Other:	D.-M. Chuang	Chemist	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology and Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the biochemistry of cholera toxin and to relate the biochemistry with activation of adenylate cyclase.

PHS-6040
(Rev. 10-76)

Project Description:

Cholera toxin is a non-reversible activator of adenylate cyclase. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we have attempted to study the biochemical mechanisms that might come into play when adenylate cyclase is activated by the toxin. We previously reported that cholera toxin hydrolyzes nicotinamide adenine dinucleotide (NAD) and that adenosine diphosphate-ribose (ADP-ribose) is incorporated into the A subunit of the toxin (Z01 MH 01519-02 SMRP). Our current objective was to determine whether cholera toxin could transfer ADP-ribose to other protein acceptors. Interferon has been reported to have several characteristics in common with cholera toxin, we therefore compared some of its biochemistry with that of cholera toxin.

Cholera toxin or interferon was incubated together with radioactive NAD and a potential protein acceptor for ADP-ribose. Radioactivity incorporated into protein was determined in an acid insoluble precipitate of the incubation mixture.

We found that proteins with similar primary and secondary structure with cholera toxin were ADP-ribosylated. These proteins include: thyrotropin, lutropin, follitropin and human chorionic gonadotropin. Interestingly, these substances all activate specific adenylate cyclases. Moreover, we found that corticotropin (1-24) and the chromatin associated proteins histone subfraction f_1 and protamine also acted as acceptor proteins for toxin mediated ADP-ribosylation. The cholera toxin catalyzed ADP-ribosylation appeared to be a function of the toxin A subunit. Interferon catalyzes the hydrolysis of NAD and we found that it also transfers ADP-ribose to protamine. Mock interferon or boiled interferon were enzymatically inactive.

In vivo ADP-ribosylation of proteins has been implicated in DNA synthesis, DNA repair, control of cell proliferation, chromatin organization and histone transport into the cell nucleus. Whether ADP-ribosylation is required for activation of adenylate cyclase by cholera toxin or whether interferon activates adenylate cyclase remains to be studied. Cholera toxin and interferon may provide the necessary pharmacological tools for understanding the coupling of adenylate cyclase system with molecules that activates the enzyme as well as the importance of ADP-ribosylation of proteins.

Our goal for the future is to understand the mechanisms by which cholera toxin activates the adenylate cyclase systems.

Publications:

Neckers, L. M., Neff, N. H., Garrison-Gund, C. K., and Wyatt, R. J.: Increased adenylate cyclase activity and rapid weight-loss following intraseptal injection of cholera toxin. *Eur. J. Pharmacol.* 51: 141-144, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01520-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The dynamics of catecholamine metabolism in the CNS: Biochemical pharmacology of the spinal cord dopaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Gentleman	Pharmacologist	SMRP	NIMH
	M. Parenti	Visiting Fellow	SMRP	NIMH
Other:	Z. Lackovic	Visiting Associate	SMRP	NIMH
	J. W. Comissiong	Visiting Associate	SMRP	NIMH
	F. Karoum	Staff Fellow	SMRC	NIMH
	S. O. Kayaalp	Visiting Scientist	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 2.8	PROFESSIONAL: 2.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the metabolism of catecholamines in brain and spinal cord and to evaluate the factors that influence amine metabolism. Our present interests are to determine whether dopamine containing neurons are present in the spinal cord and to provide information about their biochemical properties.

PHS-6040
(Rev. 10-76)

Project Description:

Our laboratory has previously provided evidence that dopaminergic neurons are present in spinal cord (Z01 MH 01520-02 SMRP). Our current objective was to determine the origin of the dopaminergic neurons and to biochemically characterize the receptors for dopamine in the cord.

The catecholamines and their metabolites were analyzed by gas chromatography-mass spectrometry. Receptor mechanisms were assessed from the ability of dopamine to activate adenylate cyclase and from the binding of a radioactive dopamine antagonist to spinal cord membranes. The origin of the spinal cord dopaminergic neurons was evaluated by lesioning or electrically stimulating selected regions of brain.

The origin of some spinal cord dopaminergic neurons

The dopamine content of striatum and thoracic spinal cord was significantly reduced on the left side when 6-hydroxydopamine (a selective neurotoxin for catecholamine-containing neurons) was injected into the left substantia nigra. In contrast, the norepinephrine content in the cord of these rats was unaltered. This study provides evidence that dopamine is present in axons that are different from those that contain norepinephrine and that some of the dopaminergic axons originate in substantia nigra and enter the spinal cord uncrossed. In support of this notion, unilateral electrical stimulation of the substantia nigra resulted in a accumulation of 3-methoxy-4-hydroxyphenylacetic acid (HVA) in the striatum and in the thoracic cord on the stimulated side. Apparently some of the dopaminergic neurons originate in the substantia nigra and enter the thoracic cord uncrossed.

Catecholamine formation in the cord

The dorsal and ventral horns of the cervical and lumbar segments and the zona intermedia from the thoracic segment of the rat spinal cord were analyzed for dopamine, norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG). Norepinephrine was rather uniformally distributed throughout the cord, whereas MHPG was higher in the ventral and zona intermediate than in the dorsal horns. Dopamine was higher in the dorsal horns and zonal intermedia and DOPAC content followed in parallel with dopamine content. The alpha-methyltyrosine method for estimating catecholamine formation revealed that norepinephrine formation was similar in all regions studied. In contrast, dopamine formation was significantly faster in the zona intermedia than in other parts of the cord. The more rapid formation of dopamine in the zona intermedia suggests that dopamine plays a key role in modulating the activity of preganglionic cholinergic input to sympathetic ganglia.

Characteristics of dopamine receptors in the cord

Dopamine-activated adenylate cyclase was found in all segments of the cord with the highest specific activity in the lumbar region. Activation by dopamine was blocked by haloperidol but not propranolol. Transsection of the spinal cord at the mid thoracic region resulted in supersensitivity of adenylate cyclase to dopamine below the lesion.

Binding sites for spiroperidol, a dopamine antagonist, have been identified in spinal cord. Binding is reversed by dopamine or (+) butaclamol a neuroleptic drug.

Identification of dopamine and its metabolites in spinal dorsal nerve roots

Following a transsection of the cord, dopamine content falls but it can still be detected below the transsection. Recently dopamine and HVA have been identified in dorsal spinal nerve roots by gas chromatography-mass spectrometry. Whether this dopamine system is entering or leaving the spinal cord via the dorsal nerve roots remains to be established. Afferent dopaminergic neurons might explain the presence of dopamine in the cord below a transsection.

Schizophrenia has been postulated to be the consequences of excessive dopaminergic influence on brain function. This hypothesis is a result of the observation that neuroleptic drugs block dopamine receptor sites. These same drugs also cause severe motor side effects. Apparently parkinsonism is the consequence of a loss of dopaminergic neurons from the nigrostriatal system. Drugs that are used to treat this disorder often cause motor abnormalities as well. Our current studies are providing the basic information needed to understand normal physiological mechanisms in addition to the side effects and neurotoxicity induced by these two important classes of therapeutic agents.

Our future studies will be directed towards evaluating the interaction of drugs with the catecholamine systems of spinal cord with special emphasis on understanding the mechanism of action of these drugs.

Publications:

Commissiong, J. W., Galli, C. L., and Neff, N. H.: Differentiation of dopaminergic and noradrenergic neurons in rat spinal cord. J. Neurochem. 30: 1095-1099, 1978.

Commissiong, J. W., Hellstrom, S. O., and Neff, N. H.: A new projection from locus coeruleus to the spinal ventral columns: Histochemical and biochemical evidence. Brain Res. 148: 207-213, 1978.

Commissiong, J. W., Gentleman, S., and Neff, N. H.: Spinal cord dopaminergic neurons: Evidence for an uncrossed nigrospinal pathway. Neuropharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01521-04 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Functional role of substance P in nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH
	J. S. Hong	Staff Fellow	SMRP	NIMH
	S. E. Robinson	Staff Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

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TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to study the functional role of substance P in substantia nigra, we have studied whether the content of substance P in nigra changes when the function of dopamine neurons is perturbed. Chronic treatment of rats with haloperidol resulted in a significant decrease of substance P concentration in substantia nigra; in contrast, the substance P content of other brain regions such as striatum and hypothalamus was not affected. This finding suggests that dopamine neurons either directly or indirectly may be involved in regulating substance P content in ths substantia nigra. A decrease in the substance P content of substantia nigra occurred only after repeated haloperidol injection, but not after a single injection, indicating the rate of substance P utilization must exceed the synthetic capability of the system. Perhaps a large pool of substance P precursor may be operative. The chick embryo dorsal root ganglion contains a molecularly heterogeneous pool of substance P-like peptides including a high molecular weight species which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with nerve growth factor. Substance P, and its apparent precursor have also been found in superior cervical ganglia, probably located in interneurons.

PHS-6040
(Rev. 10-76)

Project Description:

It has been suggested that substance P is a putative transmitter in the axons of bipolar cells which function as primary sensory neurons innervating spinal cord. Though the functional role of substance P in the brain is not clear, it can be suggested that strio-nigral and habenula-interpeduncular pathways contain substance P. In order to learn more about the interrelationship of substance P with other transmitters, i.e., dopamine, we have studied the effects of antipsychotic drugs, endowed with dopaminergic receptor blocking activity, on the concentration of substance P in substantia nigra and other brain regions.

A single injection of a high dose of haloperidol (2 mg/kg) failed to change the substance P concentration of substantia nigra. However, repeated administrations of haloperidol (1 mg/kg/day) during one week caused a 30% reduction of the substance P concentration of substantia nigra. A longer treatment caused a greater decrease in the peptide concentration of substantia nigra (a 40% reduction after two weeks of treatment). In order to understand the mechanism involved in the action of haloperidol on the concentration of substance P in substantia nigra, we have examined the content of this peptide in various brain regions. Rats receiving haloperidol for three weeks (1 mg/kg/day) exhibited a 40% reduction of substance P content in substantia nigra, whereas the substance P content of the hypothalamus and striatum remained unaltered. Hence, the haloperidol action on brain substance P content appears to be circumscribed to the substantia nigra. The effects of pimozide and clozapine on the substance P content of substantia nigra were examined. The concentration of substance P decreased after three weeks of treatment with pimozide (1.5 mg/kg/day i.p.), but failed to change after three weeks of treatment with clozapine (10 mg/kg/day i.p.). It is important to note that unlike pimozide and haloperidol, clozapine elicits little or no extrapyramidal side effects.

This study has demonstrated that there is a significant decrease of nigral substance P content following a protracted treatment with haloperidol. Whether or not this action of haloperidol on substance P content relates to the extrapyramidal side effects of haloperidol still remains to be elucidated. However, this study has suggested that a dopaminergic mechanism participates in the regulation of substance P steady-state of substantia nigra.

Lesion studies suggest that substance P may affect serotonin transmission in the dorsal raphe. Lesions of the habenula result in a 50-80% decrease of the substance P content of the dorsal raphe with no change in median raphe substance P content, thereby suggesting a peptidergic innervation of the dorsal raphe by cell bodies located in the habenula nuclei.

Evidence is accumulating that many peptides, protein hormones, and transmitters are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important regulatory point. We have used chick embryo dorsal root ganglia, which contain substance P cell bodies, to look for a precursor to substance P. Incubation of the ganglia in vitro for 20-24 hrs resulted in an increase in the concentration of substance P which was blocked by cycloheximide. In addition, incubation of 9 day chick embryo ganglia with β-nerve growth factor resulted in a further increase in the substance P content of the ganglia, an increase also prevented by cycloheximide. Gel chromatographic analysis of extracts revealed the presence of 2 peaks of substance P-like immunoreactivity (SPLI), one of which eluted at the same position as authentic substance P. The other peak, larger than substance P, was increased following NGF treatment. We believe this peak may be a precursor to substance P.

A survey of other peripheral tissues revealed the presence of substance P in sympathetic ganglia and in submaxillary gland. The substance P content of superior cervical ganglion was not affected by decentralization, section of the carotid sinus nerve or deafferentation, suggesting that it is present in interneurons. Chromatographic analysis revealed the presence of 2 peaks of SPLI, comparable to those in sensory ganglia. The substance P content of submaxillary gland did not arise from sympathetic innervation of the gland. However, part of it was associated with the chorda tympani and the rest appeared to be present in the gland cells of the submaxillary gland. Since substance P is a sialogogic compound, these results suggest that the substance P in the submaxillary gland may be involved in the stimulation of salivation.

Publications:

Hong, J. S., Yang, H.-Y. T., and Costa, E.: Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. Neuropharmacology 17: 83-85, 1978.

Schwartz, J. P., and Costa, E.: Nerve growth factor-mediated increase of the substance P content of chick embryo dorsal root ganglia. Brain Res. 170: 198-202, 1979.

Robinson, S. E., Schwartz, J. P., and Costa, E.: Substance P in the superior cervical ganglion and the submaxillary gland of the rat. Brain Res., in press.

Neckers, L. M., Wyatt, R. J., Speciale, S. G., and Schwartz, J. P.: Substance P afferents from the habenula innervate the dorsal raphe nucleus. Exp. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01524-04 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Modulation of dopamine metabolism in the sympathetic ganglion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Karoum	Staff Fellow	SMRC	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine is present in a special type of cell in sympathetic ganglia. The cells are known as small intensely fluorescent cells (SIF cells) because of their appearance after histochemical staining for catecholamines. These cells modulate information transfer from preganglionic neurons to postganglionic neurons. Our present objective was to evaluate the content of 3,4-dihydroxyphenylacetic acid (DOPAC) in the celiac ganglion as a biochemical indicator of SIF cell participation in ganglionic transmission.

Project Description:

Dopamine, released from special cells in sympathetic ganglia known as SIF cells, may function as a transmitter or modulator for slow inhibitory post synaptic potentials. The 3,4-dihydroxyphenylacetic acid (DOPAC) content of the ganglia is directly related to the formation of dopamine. It may be possible, therefore to monitor the participation of the SIF cells in ganglionic transmission by analyzing the content of DOPAC in the ganglion. Our objective was to evaluate whether a variety of drugs that are known to interact with receptors might alter dopamine metabolism in sympathetic ganglia, thus, providing circumstantial evidence that they might alter ganglionic transmission.

Dopamine and its metabolites were assayed in rat celiac ganglia by mass fragmentography. Drugs were administered parenterally.

Oxotremorine increased while atropine prevented the increase of DOPAC in the ganglion. Atropine treatment alone decreased DOPAC levels. These studies together with studies in the literature are consistent with the view that muscarinic receptors play a major role in controlling SIF cell dopamine metabolism. The beta-adrenergic receptors agonist, isoproterenol, had no effect on the content of DOPAC in the celiac ganglion. In contrast, the alpha-adrenergic receptor agonist, phenylephrine, decreased the content of DOPAC and prior treatment with phenoxybenzamine prevented the fall of DOPAC. Moreover, phenylephrine could partially antagonize the rise of DOPAC after oxotremorine treatment. The administration of the dopamine agonist apomorphine had no effect on the content of DOPAC, but the dopamine antagonists haloperidol, administered alone significantly increased DOPAC. The increase was not blocked by administering haloperidol together with apomorphine, but it appeared to be blocked by treatment with atropine. Phenylephrine treatment reversed the elevated levels of DOPAC induced by haloperidol as well. Our studies suggest that drugs that alter the DOPAC content in ganglia might be identified for further electrophysiological studies of ganglionic transmission.

Schizophrenia is associated with abnormal metabolism of dopamine. The objective of our studies is to provide a rational basis for pharmacologically modulating dopamine formation. Sympathetic ganglia with their associated dopamine containing SIF cells may provide a relatively simple model for such studies.

In the future radioactive ligands will be used to directly establish the presence of receptors binding sites on sympathetic ganglia.

Publications:

Lutold, B. E., Karoum, F., and Neff, N. H.: Activation of rat sympathetic ganglia SIF cells dopamine metabolism by muscarinic agonist. Eur. J. Pharmacol. 54: 21-26, 1979.

Lutold, B. E., Karoum, F., and Neff, N. H.: Deficient dopamine metabolism in the celiac ganglion of spontaneous hypertensive rats. Circulation

Research 44: 467-471, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01525-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of nerve growth factor content of nervous system-derived tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 1.6	PROFESSIONAL: 0.7	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nerve growth factor (NGF) is synthesized and secreted by two cell lines derived from the nervous system, the rat C6 glioma and the mouse NB2a neuroblastoma. The content of the NGF is increased in either cell line by agents which elevate cyclic AMP, β-adrenergic receptor agonists for the glioma cells and prostaglandin E_1 or adenosine for the neuroblastoma. The increase in NGF content follows an elevation of cyclic AMP content and the concomitant activation of protein kinase. The increase in cyclic AMP does not depend on an increase in the net synthesis of NGF, but rather appears to derive from an activation of a peptidase which may form NGF from a pro NGF. Research is being carried out to determine 1) the characteristics of the glioma cell protein kinase(s); 2) the precise mechanism by which protein kinase activates the peptidase and 3) the biochemical characteristics of the precursor NGF.

Project Description:

Two nervous system-derived cell lines, the C6 glioma and NB2a neuroblastoma, synthesize and secrete nerve growth factor. The cells are grown in monolayer culture using standard tissue culture methods. β-NGF has been measured by two different assays: a radioimmunoassay utilizing ^{125}I-NGF and antibody to β-NGF, and a bioassay which measures the outgrowth of neurites of dorsal root ganglia from 9-10 day chick embryos. We now have in addition two assays for measuring the α-subunit or peptidase of the large 7s NGF complex: a radioimmunoassay and an enzymatic assay using an artificial substrate.

The content of β-NGF in both cell lines, as well as the amount released to the medium, can be increased by treating the cells with agents which activate adenylate cyclase. Incubation of the glioma cells with a β-adrenergic receptor agonist causes an elevation of the cellular cyclic AMP content that is maximal in 15 minutes. Cyclic AMP-dependent protein kinase is maximally activated in 30-60 minutes, and then returns to basal activity by 3 hrs. The glioma cells contain both a cyclic AMP-dependent and independent protein kinase. The cyclic AMP-dependent form has been characterized as a Type II protein kinase on the basis of its elution pattern from a DEAE-cellulose column using a NaCl gradient.

Characterization of the protein kinase is the first step in determining how the cyclic AMP-activated protein kinase is causing an increase in the β-NGF content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. Purified nuclei from the glioma cells contain a protein kinase active against endogenous substrates which was not activated by the addition of cyclic AMP, or of calcium in the presence or absence of the calcium ionophore A23187. By 45-60 min following treatment of the cells with isoproterenol, there is a 60-300% increase of nuclear protein kinase activity. The increased activity can be inhibited by the Type I protein kinase inhibitor specific for cyclic AMP-dependent protein kinase, whereas the activity of control nuclei cannot, supporting the concept that it is the catalytic subunit of the cyclic AMP-dependent protein kinase which is translocated into the nucleus following isoproterenol treatment.

One consequence of isoproterenol activation of adenylate cyclase in the glioma cells is an induction of cyclic AMP phosphodiesterase (PDE), a process which reaches a peak in 3-4 hrs and requires new protein synthesis. Pretreatment of the cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymeization, the results suggest that microtubules are involved in the translocation process.

However, neither cycloheximide nor vinblastine affect the increase in β-NGF content elicited by isoproterenol, suggesting that at least the early (3-6 hr) increase in NGF is not the result of new protein synthesis. We believe that this increase is the result of the activation of a process whereby pro-β-NGF is converted into NGF. We are currently in the process of isolating pro-β-NGF from mouse submaxillary gland in order to examine more closely the role that the peptidase subunit of 7s NGF plays in the conversion process and the role of the cyclic AMP-dependent protein kinase in activating this process.

Publications:

Schwartz, J. P., and Costa, E.: Regulation of nerve growth factor content in a neuroblastoma cell line. Neuroscience 3: 473-480, 1978.

Schwartz, J. P., and Costa, E.: The role of cyclic AMP-dependent protein kinase in the catecholamine-mediated regulation of nerve growth factor contained in C6 glioma cells. Proc. of 4th Internat. Catecholamine Symposium, Asilomar, 1978.

Schwartz, J. P., and Costa, E.: Protein kinase translocation following β-adrenergic receptor activation in C6 glioma cells. J. Biol. Chem., sumitted.

Schwartz, J. P., and Costa, E.: Blockade by vinblastine of C6 glioma cyclic AMP phosphodiesterase induction elicited by isoproterenol. J. Pharmacol. Exp. Ther., submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01526-03 SMRP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Modulation of tyrosine hydroxylase activity in retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Iuvone	Guest Worker	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the physiological mechanisms that are responsible for modulating tyrosine hydroxylase activity in the retina. This enzyme is apparently rate-limiting for catecholamine biosynthesis. Our present interest is to determine whether tyrosine hydroxylase activity of retina is modulated by light.

PHS-6040
(Rev. 10-76)

Project Description:

Dopamine, a putative neurotransmitter in the central nervous system of mammals, is contained in a subpopulation of retinal amacrine neurons. Secretion of dopamine from these neurons is stimulated by light (see Z01 MH 01526-02, SMRP). Exposure of rats to room light results in a rapid increase in the rate of dopamine formation in retina, which persists for 4 days if the light remains on for this period. Continuous exposure of rats to light gradually increases the apparent Vmax of retinal tyrosine hydroxylase. Our objective was to determine the mechanisms responsible for the increase of the Vmax.

Rats were exposed to constant light or dark for 4 days and the kinetics of tyrosine hydroxylase was evaluated by standard procedures. Antibody to rat adrenal tyrosine hydroxylase was produced in rabbits for immunotitiation studies.

Exposure of rats to continuous light for 4 days increases the appparent Vmax of tyrosine hydroxylase but not the Km for the cofactor. Immunotitiation procedures indicate that the elevated Vmax following prolonged exposure to light is not associated with an increase of immunoprecipitable tyrosine hydroxylase molecules. Our studies, however, are consistent with the notion that the Vmax increase is a consequence of increased specific activity of the enzyme.

Retinal amacrine neurons have dendritic processes but not axons. The dendrites synapse with other amacrine dendrites, with ganglion cell dendrites and with bipolar cell terminals. Our results are consistent with the notion that an increase of the specific activity of tyrosine hydroxylase may be one of the mechanisms responsible for modulating catecholamine synthesis in dendrites. The retinal dopaminergic system offers a simple model for evaluating the biochemical processes that are responsible for the physiological activation of tyrosine hydroxylase. Only by understanding normal physiological mechanisms will it be possible to understand the pathophysiology and the mechanism of action of drugs used to treat diseases. Moreover, our studies are providing basic information on the biochemical processing of visual information.

Our future studies will be directed toward evaluating the mechanisms for the activation of the enzyme by light and drugs such as the neuroleptics.

Publications:

Iuvone, P. M., Galli, G. L., Garrison-Gund, C. K., and Neff, N. H.: Light stimulates tyrosine hydroxylase and dopamine synthesis in retinal amacrine neurons. Science 202: 901-902, 1978.

Iuvone, P. M., Galli, C. L., and Neff, N. H.: Retinal tyrosine hydroxylase: Comparison of short-term and long-term stimulation by light. Mol. Pharmacol. 14: 1212-1219, 1978

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01527-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of endogenous opioids using high pressure liquid chromatography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

High pressure liquid chromatography (HPLC) is a new technology now being developed for separation, partial purification and quantification of peptides. Work in this lab has focussed on achieving optimal high resolution separations of opiate peptides from other closely related compounds of biological origin. Results have shown that retention of peptides containing up to 20 amino acids on reverse phase HPLC columns can be predicted on the basis of amino acid composition, and that chromatographic conditions of pH and solvent composition can be decided on the basis of simple calculations. The results will be of use in any study which requires isolation of peptides for purpose of identification, or proof of purity and accuracy of measurement.

PHS-6040
(Rev. 10-76)

Project Description:

Research into the role of endogenous opioid peptides (enkephalins) and other neuropeptides and small proteins in brain function during normal and disease states requires the availability of methods that are precise, sensitive and of high specificity. No currently available technique for measuring peptides meets all these requirements. Radioimmunoassay, for example, which has been used with much success in studies of peptides in brain and other tissues because of its great sensitivity, has serious problems of specificity, due to the molecular heterogeneity of peptides. One way to achieve high specificity is to utilize a technique which allows actual physical separation of closely related compounds. Ideally, the technique should be capable of completely separating the compounds of interest from all other compounds. In that case, direct measurement of the amount of the compound is made possible. However, even if the compound cannot be completely separated from all other compounds, separating the compound first, and then using another method of different specificity gives greater specificity than either technique alone. High pressure liquid chromatography (HPLC) is a nearly ideal separation method to study opiate like compounds since it is rapid and it provides high resolution and requires small samples. Such a technique is needed for 1) confirmation of the identity of opioid compounds when these are to be measured with routine techniques that are easy, but of lower specificity; 2) isolation from brain of opiate like compounds for subsequent identification and structural determination; and 3) separation of opiate peptides from their closely related precursors and metabolites in order to study the turnover (dynamics) of these compounds. The latter is of importance to establish neuropeptide participation in drug actions or to a given behavioral output.

The OBJECTIVES of this study was to devise HPLC (high pressure liquid chromatography) methodology which would allow simple, reproducible and predictable separation of various neuropeptides.

The METHODOLOGY EMPLOYED in these studies was reverse phase HPLC. Initial experiments focussed on what kind of buffer solutions (the "mobile phase") was needed to elute the peptides from the HPLC column in high yield and that permitted high resolution separation without impairing possibilities for detection. In subsequent studies, these newly discovered conditions were used for chromatography of a variety of biologically important naturally occurring peptides: 16 different endogenous opiates, neurotransmitter candidates, endocrine releasing factors etc, and over 30 model di and tri peptides.

MAJOR FINDINGS

1) In order to increase the resolution of HPLC to separate closely related neuropeptides certain salts are added: NaC104 (at pH 2 or 7); NaH2P04 (at pH 2 but not pH 7) or 0.4 M HC104 (pH 1). Of these salts, only the NaC104 is suitable for both separation and detection of the peptides.

2) Linear gradients of acetonitrile containing one of the above salts elutes peptide from HPLC columns with optimum peak shape on reverse phase HPLC

columns.

3) The study of the retention characteristics of these peptides revealed a pattern of increased retention times with increased lipophilicity of the individual amino acids that make up the peptide.

4) Computer based calculations were made of the correlation of retention with the lipophilicity of the component amino acids. It was found that these values could be used to predict retention times with extremely high confidence (r=0.9991).

The SIGNIFICANCE of these findings for the progress of neuroscience depends on the recent finding that numerous peptides work as neuromodulator and thereby participate in the action of drugs used in psychiatry and in the etiology of mental disturbances. Other workers will be able to take the data collected (in preparation for publication) and easily design separation conditions. In this lab, these findings will open up several new areas for research. First, the high resolution made possible will allow studies of whether the transmitter peptide substance P exist in several closely related sizes -- the full 11 amino acid molecule, or an 8 amino acid molecule which is even more potent and may occur in tissue. Secondly, the high resolution we have now obtained makes it worthwhile to develop high sensitivity detection systems. Initial experiments along these lines suggest that 10 ng of enkephalins can be chemically separated from possible contaminants and detected easily. If this research line proves successful, a whole new era of precise direct measurement in studies of neuropeptides will begin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01530-03 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regional distribution and regulation of enkephalin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Hong	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Preclinical Pharmacology

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Molecular Neurobiology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To study the possible involvement of [met^5]-enkephalin (ME) in psychiatric disorders, we studied the effects of antipsychotic drugs and electroconvulsive shock (ECS) on ME content in various rat brain regions. Chronic treatment with haloperidol not only increases the ME content in striatum and N. accumbens but also elevates the content of ME-like peptide which may function as the precursor of ME. The effect of haloperidol on ME system may be mediated through the cholinergic neurons in striatum since scopolamine reduced greatly haloperidol-induced increase in ME content. This notion is further supported by the finding that chronic administration of DFP increases striatal ME content. Repeated ECS cause an increase in ME content in hypothalamus and some limbic areas such as N. accumbens, septum and amygdala. The temporal characteristics of this increase resembles the time course of the clinical effects of ECS suggesting the possibility that the ME increase may participate in the antidepressive action of ECS. Finally it was demonstrated that prolonged recurrent seizures may cause a selective increase in the production of hippocampal ME.

PHS-6040
(Rev. 10-76)

Project Description:

Previous studies from this laboratory revealed that the content of [met^5]-enkephalin (ME) in rat striatum and nucleus accumbens increases after chronic administration of antipsychotic drugs such as haloperidol. In an attempt to determine the more detailed mechanism and the neuronal circuitry underlying the haloperidol-induced increase in ME content in striatum, we studied: 1) whether chronic haloperidol treatment affect the content of the possible precursor of ME. 2) The effects of cholinergic drugs on the haloperidol-induced increase in ME content.

Two ME-like peptides with molecular weight larger than ME were detected in both bovine and rat caudate. The study on the chemical nature of these two peptides revealed that they may contain the ME sequence because they generate ME when treated with trypsin but they differ from β-lipotropin, α- or β-endorphins. Daily injections of haloperidol for 3 weeks increase not only the ME content but also the content of the ME-like peptide which was eluted close to the void volume in a Biogel P-2 column chromatography (MW >1800). Since under this condition haloperidol failed to cause a non-specific increase in protein synthesis (based on the rate of incorporation of [^{3}H] leucine into protein precipitable with hot TCA), the concomitant increase in ME and high molecular weight ME like peptide content after haloperidol, further supports the possibility that ME and this ME-like peptides may be interrelated.

To explore the possibility that the transsynaptic regulation of striatal ME neurons by dopaminergic terminals may be mediated through cholinergic interneurons, cholinergic drugs were employed. Scopolamine greatly reduced haloperidol-induced increase in ME content. Furthermore, chronic adminstration, but not a single injection of DFP (an anticholinesterase inhibitor), caused a significant increase in striatal ME content. These two studies suggest that cholinergic neurons may participate in the neuronal loop that regulates transsynaptically ME neurons.

After demonstrating that chronic treatment with haloperidol increased ME content, we explored whether the content of brain ME is changed by electroconvulsive shock (ECS) which has been the major treatment for refractory depressive psychoses. Repeated electroconvulsive shocks (ECS) cause an increase of ME content in hypothalamus and some limbic areas such as N. accumbens, septum and amygdala. The temporal characteristics of this increase resembles the time course of the clinical effects of electroconvulsive therapy (ECT). Single shock failed to change the ME content in any part of the brain. After 6 daily shocks ME content increases by 60% in hypothalamus and maximal effect (100% increase) was obtained after 10 daily shocks. The increase of ME content persists 6 days after the termination of ECS. Subconvulsive shocks failed to alter the content of ME in all the brain regions determined. When the seizure induced by ECS was prevented by pretreatment with an anesthetic dose of phenobarbital, the ECS induced increase in hypothalamic and septal ME content was also blocked. Beta-endorphin content of hypothalamus remained unchanged after repeated ECS despite a two fold increase in the ME content in the same region. These observations indicate that the time constant for the increase in ME content induced by ECS resembles the time constant for the appearance of the clinical benefits by ECT; this coincidence may give credence to the possibility that the ME increase may participate in the antidepressive action of ECS.

As an extension of the study on the regulation of brain ME neurons, we studied the regulation of hippocampal ME content following recurrent motor seizures.

Unilateral intrahippocampal injection of kainic acid (K.A.) (1 μg in 1 μl of 0.9% NaCl) produced recurrent motor seizures lasting about 5 hr which were associated with a selective change in the ME content of hippocampus. Six to 12 hr after injection, there was a small decrease in ME content in both injected and contralateral hippocampus, however within 24 hr after the injection of ME content was increased. The elevation in the injected hippocampus reached a maximal level (200%) after 2 or 3 days, this plateau lasted longer than 14 days. In contrast the ME content of the contralateral side increased by a greater extent reaching a peak at day 2 (300%) but returned to control level by day 14. The increase in ME content was greatly reduced when cycloheximide was administered intraventricularly 6 hr after intrahippocampal injection of K.A. This result suggests that the increase in ME content elicited by K.A. injection is probably due to an increase in the synthesis of ME rather than to a decrease in the efflux of ME from the hippocampus. The selective increase in hippocampal ME content was also elicited by intraseptal, intrastriatal or intraventricular injection of K.A. Since all the procedures caused recurrent motor seizures it is suggested that the change in hippocampal ME content after K.A. may be related to the recurrent seizures elicited by K.A. In fact the increase in ME content after intraseptal injection of K.A. was blocked when muscimol, an anticonvulsant, was injected together with K.A. Moreover, the increase in hippocampal ME 2 days after recurrent seizures was also induced by isoniazid or by repeated electroconvulsive shock (4 shocks given at 30 min intervals). Thus, it appears that prolonged recurrent seizures may cause a selective increase in the production of hippocampal ME. The significance of the participation of ME in this convulsive state remains to be elucidated.

Publications:

Yang, H.-Y. T., Hong, J. S., Fratta, W., and Costa, E.: Rat brain enkephalins: Distribution and biosynthesis. In Costa, E. and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, pp. 149-159, 1978.

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Hong, J. S., Yang, H.-Y. T., Gillin, J. C., Fratta, W., and Costa, E.: Participation of [met^5] enkephalin in the action of antipsychotic drugs. In Usdin, E. (Ed.): Endorphins in Mental Health Research. London, England, Macmillan Press, pp. 105-114, 1979.

Yang, H.-Y. T., Hong, J. S., Fratta, W., and Costa, E.: An approach to assess the dynamics of met^5-enkephalin storage in rat striatum. In Usdin, E. (Ed.): Endorphins in Mental Health Research. London, England, Macmillan Press, pp. 235-241, 1979.

Hong, J. S., Yang, H.-Y. T., Gillin, J. C., Di Giulio, A. M., Fratta, W., and Costa, E.: A chronic treatment with haloperidol accelerates the metabolism of enkephalins in rat striatum. Brain Res. 160: 192-195, 1979.

Hong, J. S., Yang, H.-Y. T., Gillin, J. C., and Costa, E.: Effects of long term administration of antipsychotic drugs on enkephalinergic neurons. In Proceedings of the International Symposium on Neuroleptics, Monte-Carlos, Monaco, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01531-02 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Nerve growth factor in human fibroblasts from controls and familial dysautonomia patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	E. Costa	Chief	SMRP	NIMH

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TOTAL MANYEARS: 0.2
PROFESSIONAL: 0.2
OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Skin fibroblasts from human controls or from patients with familial dysautonomia (FD) have been grown in tissue culture. β-Nerve growth factor has been measured in extracts from both type of cells, using a radioimmunoassay and a bioassay. The FD cells have contents of NGF as measured by RIA comparable to the controls. However, the biological activity of the FD samples is about one-tenth that of the controls when expressed as biological units of activity per ng β-NGF as measured in the RIA. Stimulation of β-adrenergic receptors in both cell lines with l-isoproterenol results in a 17-170 fold rise in intracellular cyclic AMP in 10 min. The NGF content of the control fibroblasts increased 50-300% after 3-4 hrs of exposure to isoproterenol whereas there was no change in the NGF content of the FD cells at any time.

PHS-6040
(Rev. 10-76)

Project Description:

Familial dysautonomia (Riley-Day Syndrome) is an autosomal recessive disease, in which patients have impaired autonomic and sensory functions. Symptomatically, these patients resemble animals that have been treated with antiserum to nerve growth factor. It has been suggested that there may be an impairment of nerve growth factor associated with the disease, and there are reports that FD patients have elevated serum NGF levels.

We have chosen to use skin fibroblasts from FD patients, and fibroblasts from paired controls, to study this problem further. The fibroblasts are grown in monolayer culture, using early passage cells and standard tissue culture procedures. Nerve growth factor is assayed using a radioimmunoassay which detects β-NGF as well as the β-component of 7s NGF, and also a bioassay in which neurite sprouting from 9 day chick embryo dorsal root ganglia is measured.

NGF remains essentially constant in both control and FD fibroblasts as they go from log to stationary phase growth. Control and FD cells have been found to contain comparable amounts of NGF as measured by radioimmunoassay. However, estimation of the NGF content of the same extracts by a bioassay procedure shows that the extracts from FD cells are much less biologically active than cells from paired controls. When expressed as biological units of NGF per ng β-NGF (as measured in RIA), there is a ten-fold difference in biological activity. Five FD lines and seven control lines have now been assayed.

These fibroblasts have a β-adrenergic receptor linked to adenylate cyclase. Stimulation with 1-isoproterenol caused a 17 to 170 fold increase of cyclic AMP in all lines tested. In the control lines, NGF content increased 50-300% within 3-4 hrs following isoproterenol. However there was no change in the NGF content of any FD cell line over the course of a 7.5 hr treatment with isoproterenol. These results suggest that there may be a defect in the processing of a pro-β-NGF into β-NGF in familial dysautonomia. The defect could lie in the pro-β-molecule, in the converting peptidase, thought to be the γ-subunit of 7s NGF, or at some other step. Research is now being carried on to determine what the chemical differences between the control and FD nerve growth factors are.

Publication:

Schwartz, J. P., and Breakefield, X. O.: Altered nerve growth factor in fibroblasts from patients with familial dysautonomia. Proc. Natl. Acad. Sci. USA, submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01532-02 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of catecholamine receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Chuang	Chemist	SMRP	NIMH
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	L. Farber	Chemist	SMRP	NIMH
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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	0.6

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found that in frog erythrocytes the number of beta-adrenergic receptor recognition sites is greatly increased in the cytosol fraction when the cells are preincubated with (-) isoproterenol. This increase is agonist-specific and blocked by the presence of the incubation medium of a beta-adrenergic receptor antagonist, alprenolol. Concomittant with this agonist-induced increase, the number of membrane-bound beta-adrenergic receptor sites is reduced. There is a tight correlation between these two events. Neither process requires the new synthesis of RNA or protein. We have proposed that the recognition sites of this receptor are internalized from the plasma membrane into cytoplasm during desensitization of adenylate cyclase to beta-receptor stimulation and that this receptor internalization accounts for at least part of the receptor lost from the plasma membrane. The event of internalization is absolutely temperature and energy-dependent and diminished by cordycepin, an inhibitor of cyclic nucleotide dependent and independent protein kinases. The internalized receptor is sensitive to pronase but resistant to phospholipase. Unlike the membrane-bound beta-receptors, the binding affinity of the soluble receptors for the beta-adrenergic agonists is unaffected by the presence of guanylyl nucleotides.

PHS-6040
(Rev. 10-76)

Project Description:

When the beta-adrenergic receptors on the plasma membrane of various cell types are continuously exposed to the catecholamine agonists, the responsiveness of adenylate cyclase to further receptor stimulation is greatly desensitized. Lefkowitz and coworkers have used frog erythrocyte as a model system to study the mechanism of desensitization elicited by prolonged stimulation with (-) isoproterenol. The results suggest that desensitization is directly due to a decrease in the number of recognition sites of beta-adrenergic receptors in the plasma membrane but fail to elucidate the molecular mechanisms that caused this change. Recently several laboratories have reported that many polypeptide hormones and/or their receptors are internalized from the plasma membrane following agonist binding to surface receptors. We have investigated whether internalization of beta-adrenergic receptors occurs in frog erythrocytes stimulated with (-) isoproterenol.

We have found that the 30,000 x g supernatant of frog erythrocyte lysate, possesses a binding activity for (-) [^{3}H]-dihydroalprenolol ((-)-[^{3}H]-DHA) which can be detected using Sephadex G-50 column chromatography. This [^{3}H]-DHA binding activity is enhanced 4-fold when the cytosol is derived from erythrocytes preincubated with (-) isoproterenol. This increase is prevented when cells are preincubated with the agonist in the presence of (-) alprenolol, a beta-receptor blocker, but not by phenoxybenzamine, an alpha-adrenergic blocker. Binding displacement experiments have shown that these soluble [^{3}H]-DHA binding sites have characteristics of beta$_2$-adrenergic receptors found in the frog erythrocyte membranes. Scatchard analysis revealed that the (-) isoproterenol-induced enhancement of [^{3}H]-DHA binding represents an increase in the number, but not the affinity, of soluble beta-adrenergic receptor sites. The Kd of the [^{3}H]-DHA binding to these soluble receptors is about 8 nM. This increase of soluble binding sites is time and dose dependent; the maximal increase occurs between 2 and 4 hrs after stimulation and the dose of (-) isoproterenol required for half-maximal effect is in the range of 60-90 nM. These values correspond to those required for the decrement of the number of membrane-bound beta-adrenergic receptor sites. Moreover, we found that the ability of various receptor agonists to induce this increase in the density of soluble receptors is comparable with their affinity for the beta$_2$-adrenergic receptors. Neither the treatment of cells with actinomycin D nor that with cycloheximide affects the number of basal and isoproterenol-induced soluble receptor binding sites, suggesting that new synthesis of RNA and protein is not essential for these events. We have therefore proposed that the recognition sites of membrane-bound beta-adrenergic receptors are internalized during subsensitization elicited by beta-adrenergic catecholamines.

Experiments using cells incubated with ($\pm$)-[^{3}H]-hydroxybenzylisproterenol followed by extensive washings to remove the unbound agonist have shown that the labeled agonist is internalized with the beta-adrenergic receptors. Little or no label is internalized when the (-)-[^{3}H]-DHA is used in a parallel experiment. These changes in the number of beta-adrenergic receptors in the soluble and particulate fractions are dependent on the temperature of the pre-incubation medium. Thus at 0° no change in the number of membrane receptor is observed. When the temperature is 14° or higher, significant membrane receptors begin to be internalized following incubation with isoproterenol. Concomitant with the translocation of the recognition sites from membrane to cytosol we found that the in vivo phosphorylation of membrane protein of 60,000 and 38,000 daltons is selectively increased. The doses of (-) isoproterenol for promoting the increase of membrane protein phosphorylation, receptor subsensitivity and internalization are similar. Moreover, dinitrophenol and iodoacetate, both of which diminish intracellular ATP, and cordycepin, which inhibits the cyclic nucleotide dependent and independent protein kinases, decrease the receptor internalization in a dose-dependent manner. These results suggest that membrane protein phosphorylation triggered by the receptor stimulation with isoproterenol may play a role in causing the receptor internalization.

The internalized beta-adrenergic receptor is sensitive to pronase digestion but resistant to phospholipase treatment. Most of the beta-receptors in the 30,000 x g supernatant of erythrocyte lysate remain soluble following the centrifugation at 100,000 x g for one hr. Furthermore, GTP or Gpp(NH)p does not affect the affinity of (-) isoproterenol for the soluble beta-receptors, although that for the membrane-bound receptor is decreased by one-order of magnitude. Thus the internalized receptors are protein recognition sites which are functionally "uncoupled" to adenylate cyclase.

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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Distribution and biosynthesis of beta-endorphin in brain and pituitary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	W. Fratta	Visiting Associate	SMRP	NIMH
	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The β-endorphin (β-E) immunoreactive peptides are highly concentrated in the anterior and intermediate lobes of pituitary and the hypothalamus. However a great variation is observed in the relative amount of these three β-E like materials in different regions. In the hypothalamus and the intermediate pituitary, β-E prodominates and β-LPH and pro-opiocortin are the minor components. In the anterior pituitary, the ratio of β-E/β-LPH is about 1. The result is in good agreement with the view that the pro-opiocortin may function as precursor for ACTH and β-E in the anterior pituitary and intermediate pituitary respectively. Abnormal behavior and endorphin disturbance have been reported in obese mice. In these obese mice, β-E, β-LPH and pro-opiocortin are all elevated in the pituitary but not in hypothalamus. This result raises the possibility that the abnormal β-E metabolism may be involved in development of abnormal behavior and endocrine disturbance in obese mice.

PHS-6040
(Rev. 10-76)

Project Description:

Beta endorphin, the c fragment of β-lipotropin was first isolated from the pituitary and identified to be the most potent endogenous ligand of the opiate receptor, in 1976. Since then, its highly localized distribution in pituitary and hypothalamus has been well established. Furthermore, studies on β-endorphin biosynthesis have revealed a close metabolic interrelationship between ACTH and β-endorphin, i.e. ACTH and β-endorphin are derived from the same high molecular weight precursor named 31 K ACTH/β-endorphin or pro-opiocortin. Subsequently, it has been demonstrated that β-endorphin and ACTH are concomitantly released into blood following stress or glucocorticoid administration. Since the hypersecretion of cortisol in severe depression has been well documented, it is possible that ACTH and β-endorphin systems may play a role in mental disorder. As an initial step in exploring the possible involvement of β-endorphin in mental illness, we have decided to study β-endorphin and its precursors, β-lipotropin and pro-opiocortin, in pituitary and hypothalamus. By the use of gel filtration chromatography in combination with radioimmunoassays with antisera directed against β-endorphin, β-MSH and ACTH, the existence of β-endorphin, β-lipotropin and pro-opiocortin is demonstrated in all the tissues studied with the highest concentration in the posterior-intermediate lobe of the pituitary. In addition, a great variation is observed in the relative amount of these three β-endorphin-like immunoreactive peptides in different regions. In the hypothalamus and intermediate-posterior lobe, β-endorphin predominates and a relatively small proportion of the total immunoreactivity is due to the precursors, β-lipotropin and pro-opiocortin. In the anterior pituitary, the ratio of β-endorphin and β-lipotropin is about 1 to 1 and about 20% of the total immunoreactivity is due to pro-opiocortin. A β-MSH-like immunoreactive peptide which is larger than porcine β-MSH and smaller than β-lipotropin is detected in the posterior-intermediate lobe but not in the anterior lobe of the pituitary. The results suggest that in the anterior lobe, pro-opiocortin may act mainly as a precursor of ACTH, and β-lipotropin is a metabolite. In contrast, in the hypothalamus and the intermediate lobe of the pituitary, the pro-opiocortin may act as a precursor of β-endorphin and there is active processing of β-lipotropin to β-endorphin and β-MSH like peptide.

Abnormal behavior such as overeating and overdrinking and endocrine disturbances such as hyperinsulinemia and hyperglycemia have been observed in genetically obese mice. Recently, the elevated level of β-endorphin in these obese mice has been well documented. In order to explore the role of β-endorphin in the genetically obese mice, we have studied the endorphins in brains and pituitaries of the obese mice and lean littermates. β-Endorphin, β-lipotropin and pro-opiocortin are found to be all elevated in the ob/ob mice in the pituitary but not in the hypothalamus. The result suggests an abnormal β-endorphin or ACTH metabolism in the pituitary but not in the hypothalamus of the ob/ob mice.

We are currently studying 1) the β-endorphin, β-lipotropin and pro-opiocortin in the anterior and posterior-intermediate lobes of the pituitary, 2) possible β-endorphin receptors and their physiological function(s) in the periphery, 3) the release of β-endorphin from the pituitary under various conditions such as stress, morphine treatment, etc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
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U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01534-02 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Location and role of [met^5] and [leu^5]-enkephalin and enkephalin-like peptides in neuronal tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	E. Majane	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have detected both high (HMW) and low (LMW) molecular weight enkephalin-like peptides in sympathetic ganglia and adrenal glands. In deafferented adrenal glands, the content of LMW enkephalin-like peptides is reduced, also in primary cultures of chromaffin cells contain little or no LMW but HMW forms are abundant. These results suggest that LMW forms may be located in afferent axons and the HMW forms in chromaffin cells. Conversely one can also argue that LMW forms are produced by nerve impulses. The HMW forms contain the enkephalin sequence, probably at their c-termini, these peptides may function as pro-enkephalins. Since similar LMW enkephalin-like peptides are present in blood, it is conceivable that chromaffin cells may release the LMW peptides into blood where they function as hormone. We have studied the content of HMW and LMW enkephalin-like peptides in tissues of spontaneously hypotensive rats (SHR) and found that LMW peptides are reduced in peripheral but not in brain tissue. The results suggest that enkephalin-like peptides in sympathetic ganglia and adrenal glands may play some role in regulation of vascular tone.

Project Description:

The enkephalins have a distinctive regional distribution in the brain and are also present in the peripheral nervous system. By the use of radioimmunoassays in combination with gel filtration chromatography, we have demonstrated a high degree of molecular heterogeneity in enkephalin-like peptides in several peripheral tissues such as sympathetic ganglia, adrenal galnds and ileum. Provisionally we indicate two species of such enkephalin-like peptides with high molecular weight (HMW) and low molecular weight (LMW). There are several reports which indicate that ganglia and adrenal medulla are opioid sensitive tissues, though the receptors on ganglia are naloxone insensitive in the cat and nonstereospecific in the rat. This evidence suggests that enkephalins may participate in the regulation of ganglionic transmission and adrenal medulla secretion.

By radioimmunoassays with antisera directed against met^5- and leu^5-enkephalins, we have detected enkephalin-like peptides in coeliac and superior cervical ganglia of rats, guinea pigs and rabbits. The enkephalin-like peptides were further characterized by column and thin layer chromatographic techniques and was found to consist of a pentapeptide, enkephalin, and also considerable amount of HMW (>1800 daltons) and LMW enkephalin-like material. The existence of multiple forms of enkephalin-like materials was also observed in adrenal glands of various species such as bovine, mice, guinea pigs and rats. The bovine adrenal gland is particularly enriched with the enkephalin-like materials. Both met^5- and leu^5-enkephalins were found to be highly concentrated in the medulla, and only a very small amount was detected in the cortex. In addition to the LMW enkephalin-like materials, a large proportion of the total immunoreactive enkephalin-like material is present in HMW forms which are inactive in binding to opiate receptors. Further characterization study indicates that the high molecular weight forms contain, at their c-terminal portions of structures, the sequence of enkephalin which can be released by trypsinization. The results suggest that the HMW forms may function as precursors of enkephalin. In addition to pentapeptide, other LMW forms of immunoreactive peptidds are also present in adrenal medulla. The characterization of the newly found LMW enkephalin-like material is in progress. The preliminary results suggest that this newly found LMW enkephalin-like peptide is biologically active based on its effect on blood pressure. Immunohistochemical studies have shown that enkephalin-like material is present both in afferent axons and chromaffin cells in adrenal glands. We have found that primary cultures which are devoid of innervation, contain mostly the HMW forms of enkephalin-like materials and only a trace of LMW forms. The result led us to speculate that afferent axons may contain more enkephalin than HMW enkephalin-like materials and the LMW enkephalin-like material present in chromaffin cells may be released into blood where it functions as a hormone.

Morphine is a vasodilator. Thus, it is possible that the LMW enkephalin-like material may also cause vasodilation where it is secreted. In young spontaneously hypertensive rats (SHR), work from this laboratory has shown that there is a defect in the regulation of sympathetic ganglionic transmission by endogenous dopamine. In view of a possible role of enkephalin in the regulation of sympathetic transmission and of vascular tone, we have studied the contents of enkephalins in several tissues of SHR and Wistar Kyoto (control) rats (WKY). The contents of LMW enkephalin-like peptides in coeliac and superior cervical ganglia, salivary glands and adrenals are lower in SHR and WKY at ages 8 weeks or older. No change in the enkephalin content was observed in hypothalamus. These findings suggest that the possible defect that brings about the decreased content of enkephalin in peripheral tissue of SHR may be in the enzyme that converts a HMW peptides precursors into LMW peptides or enkephalin.

We are planning to 1) characterize the LMW enkephalin-like peptides in blood and trace their origin, 2) explore the physiological role of the enkephalin-like material in chromaffin cell by studying its release and 3) study the enkephalin biosynthesis by a pulse-chase experiment.

Publications:

Di Guilio, A. M., Yang, H.-Y. T., Lutold, B., Fratta, W., Hong, J., and Costa, E.: Characterization of enkephalin like material extracted from sympathetic ganglia. Neuropharmacology 17: 989-992, 1978.

Di Guilio, A. M., Yang, H.-Y. T., Fratta, W., and Costa, E.: Decrease in the content of immunoreactive [met^5] enkephalin-like peptide in peripheral tissues of spontaneous hypertensive rats. Nature 278: 646-648, 1979.

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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Quantitative multiple ion detection mass spectrometry of neuronal peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Peralta	Guest Worker	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The applicability of mass spectrometric methods to the qualitative and quantitative analysis of peptides in biological material has been evaluated. Two general methodologies have been developed: one involves cleaving of the peptides with the enzyme Depeptidyl-amino peptidase I (D.A.P.I.) and gas chromatographic-mass fragmentographic detection of the dipeptides thus generated, the other consists in selected ion monitoring of the fragments arising from a derivative of the whole peptide molecule, using the solid inlet of the instrument as sample entry port.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective is to explore the feasibility of applying mass spectrometric techniques to the identification and measurement of peptides in biological material.

Various peptides have recently received increased attention as putative neuromodulators and neurotransmitters. Available chemical or immunohistological methods have furnished us with valuable information about the anatomical localization and possible physiological roles of neural peptides; these methods, however, cannot be regarded as totally specific and may in fact yield misleading data due to unavoidable cross-reactivity between structurally related peptides. In this context, development of an alternative, more specific, analytical technique was deemed important. Thus, the intrinsic significance of this project lies in the possibility of capitalizing on the well-established specificity of mass spectrometry, to obtain more information about the identity of neuropeptides operative in the physiology of the nervous system.

We have evaluated the advantages of the two methodological approaches centering specifically on the analysis of methionine-enkephalin (ME). The first method involves cleavage of the peptide by the enzyme D.A.P.I. as described by Krutzsch and Pisano (Meth. Exymol. 47: 391-404, 1977). In the case of ME, this yields two dipeptides (Tyr-Gly and Gly-Phe) and one amino-acid (Met). The hexafluoroisopropyl pentafluoropropionyl derivatives of these compounds are readily separated by gas chromatography in various stationary phases (OV-17, OV-1, and SE-52). Recording of the mass spectra of these derivatives assures their structural identification. After complete enzymatic hydrolysis, the ratio Tyr/Gly/Gly-Phe/Met is characteristic for ME and can be used to monitor this peptide in neural tissue. A previous purification step involving adsorption chromatography on Bio-Beads, S,-2 and thin layer chromatography has been shown to eliminate interferring compounds. We are currently extending this work to achieve quantitative analysis through the use of a deuterated ME analog as an internal standard.

The second methodology being evaluated involves analysis of the whole peptide molecule introduced into the mass spectrometer via the solids inlet. ME reacts readily with HCl-Methanol and trifluoroacetic anhydride to yield a methyl-diacyl derivative which is volatile enough to be analyzed via the solids inlet. Since this approach excludes gas chromatography, a thorough pre-purification of the biological sample appears necessary: adsorption chromatography followed by high pressure liquid chromatography is being tested for that purpose.

Biologically active peptide molecules often show striking differences in activity following minor changes in structure. Methods to detect these structural differences must therefore be extremely accurate and sensitive. The introduction of mass spectrometry for this purpose may be considered an important step forward toward the goal of absolute specificity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01536-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of receptors for putative neurotransmitters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. O. Kayaalp	Visiting Scientist	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the receptors for putative transmitter substances. Our present interests are to determine the distribution of some of the properties of the muscarinic receptors in the spinal cord and adrenal medulla.

Project Description:

We have studied the binding of radioactive quinuclidinyl benzilate (QNB), a muscarinic antagonist, to plasma membranes in order to identify muscarinic receptors. The location of these receptors might give insight into the mode of action of some cholinergic drugs. Spinal cord and adrenal medulla were chosen for evaluation because physiological and pharmacological studies reported in the literature suggested that muscarinic receptors are present in these tissues and have not been characterized.

Radioactive QNB was incubated with plasma membranes from various regions of rat spinal cord and adrenal medulla. Bound radioactivity was separated from free radioactivity by filtration.

QNB binding sites were found in both gray and white matter. The binding capacity of the former, however, was about 3-4 times higher than the latter. The area richest in muscarinic receptor binding sites was the anterior horn, with a Bmax ranging from 62 to 122 fmol/mg protein. Binding was lowest in the thoracic cord when compared with the other areas of the cord. The dissociation constant of the binding sites for QNB seemed to be comparable at different levels with Kd values ranging from 0.06 to 0.09 nM. A section of the spinal cord at the thoracic level did not change the binding parameters to a significant extent in gray and white matter above or below the section.

Muscarinic receptor binding sites were found in the rat and bovine adrenal gland associated primarily with the medulla. The Kd values found in these tissues were similar to those found for spinal cord. Moreover, denervation of the rat adrenal gland had no significant effect on the binding of QNB. Apparently the response of muscarinic receptors to denervation is quite different when compared with other neurohormone receptors. For example, there is an increase in the number of catecholamine receptor binding sites after denervation. The increased number of receptor sites is associated with receptor supersensitivity.

Abnormal cholinergic mechanisms have been proposed to explain some psychiatric and sleep disorders as well as the side effects of drugs. Our studies are providing the basis for understanding the interaction of cholinergic neurons with other neuronal systems.

Future studies will deal with the possible alteration of muscarinic binding sites by chronic treatment with drugs that apparently act at cholinergic receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01537-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of GABA receptor system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Leon	Guest Worker	SMRP	NIMH
Other:	M. Baraldi	Visiting Associate	SMRP	NIMH
	G. Toffano	Guest Worker	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

M. Massotti, FDA, Bureau of Drugs, Washington, D.C. 20204

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

GABA-modulin, an endogenous membrane protein, which inhibits non competitively ^{3}H-GABA binding to synaptic plasma membranes, has been isolated and characterized. This protein material is also a potent inhibitor of membrane protein phosphorylation and acts as a competitive inhibitor of the protein kinase substrates. The role of GABA-modulin in the control of GABA receptor system function is presently being investigated.

PHS-6040
(Rev. 10-76)

Project Description:

When GABA receptor sites are occupied by the endogenous agonist the membrane permeability to Cl^- ions changes resulting a hyperpolarization or depolarization of the receptive neurons. The goal of this project is to provide a better understanding of the function of the GABA receptor complex and in particular the coupling of GABA recognition site with Cl^- ionophore as a first step to develop new potent and specific drugs which like benzodiazepines can alter the function of the GABA receptors.

Our studies indicate that in freshly prepared crude synaptic membranes, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines, contain only one population of ^{3}H-GABA binding sites. Repeated washings of these membranes combined with freezing, thawing and treatment with Triton X-100 unmasks an additional population of GABA binding characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites the total density of GABA receptor sites and the relative portion of high (Kd 20-40 nM) and low affinity component (Kd 200-400 nM) of ^{3}H-GABA binding can be estimated.

The increase in number of high affinity binding sites for GABA after Triton X-100 treatment is most likely due to the removal of an endogenous inhibitor protein (GABA-modulin) from the membranes because the increase of ^{3}H-GABA binding is paralleled by a decrease of GABA-modulin content in these membranes. Furthermore the addition of small amounts of purified GABA-modulin to the Triton X-100 treated membranes readily masks the population of receptor with high affinity binding sites.

Using filtration procedures and ion exchange column chromatography GABA-modulin has been purified up to 1500 fold. A purified preparation of GABA-modulin applied to a SDS polyacrylamide gel electrophoresis migrates as a single protein band which contains inhibitory activity for GABA binding and for protein phosphorylation. These data suggest that the inhibitor of GABA binding is identical in nature with the Type II inhibitor of protein kinases previously described and characterized.

In agreement with this observation removal of GABA-modulin from synaptic membranes, using Triton X-100, resulted in an increase of membrane phosphorylation. The extent of this increase is similar to the increase in GABA binding observed in the same preparation. Both the increase in GABA binding and the increase in protein phosphorylation are nullified by the addition of purified GABA-modulin. These experiments indicate that GABA-modulin may interfer with the function of the GABA receptor unit perhaps by altering the state of membraine phosphorylation.

Evidence has been accumulated to suggest that GABAergic abnormalities may be related to the symptoms of Huntington's disease, Parkinson, epilepsy and possbily schizophrenia. Thus biochemical and pharmacological characterization of the GABA receptor system may be a relevant approach to find compounds which are capable of modifying the GABAergic system in man.

In future experiments we plan to develop a simple method to measure GABA-modulin content in different biological samples and to understand whether drugs that interfer with GABA-modulin action may be useful in the control of GABA receptor system in neurological or psychiatric disorders.

Publication:

Toffano, G., Guidotti, A., and Costa, E.: Purification of an endogenous protein inhibitor of the high affinity binding of GABA to synaptic membranes of rat brain. Proc. Natl. Acad. Sci. USA 75: 4024-4028, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01538-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The biochemistry of hippocampal synapses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

Mr. P. Oliver, Office of the Division Chief

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project will attempt to establish biochemical responses to, pharmacological and electrical stimulation of hippocampal slices. Stimulation of hippocampal slices with cholinergic agonists or electrical impulses applied to the fimbria. Schaffer collaterals and many fibers will be studied with regard to their ability to increase cyclic GMP content of the CA-3 region. The intracellular effect of cyclic GMP will also be examined by measuring cGMP dependent protein kinase and protein phosphorylation. This project will thereby extend and better charcterize the post synaptic sequence of events that results after stimulation of various pathways and synapses and thereby further our understanding of the events triggered by neurotransmitters as they apply to hippocampal functions.

PHS-6040
(Rev. 10-76)

Project Description:

The hippocampus is part of the cortex ideally placed to integrate signals from sensory associative areas and from sleep arousal and sensory motor areas of the brain stem. The precise mechanism of this interaction still remains an enigma. Moreover, due to its relatively simple structure and its special position at the crossroads between cortico-cortical pathways and cortico-subcortical circuits, the hippocampus has great potential as a model for cortical function.

It is our goal by using biochemical measurements and pharmacological or electrical stimulations of the hippocampus to establish the sequence of biochemical events that follow stimulation of various receptors and neuronal pathways. We will start with the cholinergic systems. Cholinomimetic agents are known to elevate levels of cyclic guanosine-3',5'-monophosphate (cyclic GMP) in certain brain areas. Thus cyclic GMP and activation of cGMP dependent protein kinase may mediate some of the effects of acetylcholine in hippocampus. We will therefore examine what effects cholinergic agents have on cyclic GMP content of various areas of the hippocampus. Each slice will be divided into CA_1, CA_2, CA_3, and CA_4. The cholinergic responses will be characterized further by comparing the effects of direct stimulation of the fimbria, a well known cholinergic input from the septal nucleus to the CA-3 region of the hippocampus. It is expected that this stimulation will increase cyclic GMP levels of the CA-3 region by using the hippocampus intrinsic cholinergic pathways and neurochemistry.

The second phase of this project will be to examine what intracellular changes that take place as a result of raising cyclic GMP levels either through pharmacological or electrophysiological manipulations. From this approach, it is expected to further define and delineate cascade of events triggered by stimulation of the cholinergic receptors at the intracellular level and thereby enrich our current understanding of hippocampal and cortical function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01539-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Different ontogenic development for soluble and membrane bound protein kinase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	F. Vargas	Guest Worker	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found an increase during development in the specific activity of cyclic AMP dependent and calcium dependent protein kinases of synaptic membrane from rat brain while the specific activity and cyclic AMP binding affinity of the soluble cyclic AMP dependent protein kinase was independent of the age of the animal. The concomitant rise in the membrane protein kinase with synaptic formation supports the concept that these enzyme are required for synaptic transmission.

PHS-6040
(Rev. 10-76)

Project Description:

Cyclic AMP and calcium dependent protein kinases are present in crude synaptic membrane preparations from brain tissues. Present evidence implicates both the cyclic AMP and the calcium dependent protein kinases with synaptic transmission. We reasoned that because of this functional linkage during ontogenesis there might be an increase in the protein kinase activity bound to synaptic membranes following synaptogenesis. In contrast, the cyclic AMP dependent protein kinase activity of cytosol would not be expected to change since this enzyme activity relates to the regulation of metabolic events which are in part or totally independent from synaptic formation and subsequent neurotransmitter release. We used synaptic membranes and cytosol from newborn and mature rat brains to study this theory.

The specific activity, DEAE elution profile and cyclic AMP binding affinity were used to characterize the soluble cyclic AMP dependent protein kinase activity. The specific activity, cyclic AMP stimulation, and calcium stimulation were used to evaluate changes in membrane bound protein kinase activity.

We found no change in specific activity, DEAE elution profile or cyclic AMP affinity of the newborn versus mature cytosol. The membrane found protein kinase specific activity stimulated by cyclic AMP was 30% higher and by calcium was 236% higher in mature synaptic membranes.

Much evidence now supports the interrelationship that neurotransmitters, cyclic AMP and protein kinase play in synaptic transmission. The interdependence is further demonstrated developmentally by previous reports of postnatal increases in the levels of cyclic AMP by adenyl cyclase and activities of cyclic nucleotide phosphodiesterase and extended here with protein kinases. All of these enzymes accompany synaptogenesis thereby providing the mechanism to allow synaptic transmission.

This concept is presently being tested with the use of hippocampal synaptic membrane and specific lesions of efferent structure.

Publication:

Kinnier, W. J., Chikvaidze, V., Hollenbeck, R. A., Chuang, D.-M., and Costa, E.: A different development for soluble and membrane bound protein kinases of rat brain. Developmental Neuroscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01540-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Newly detected biologically active enkephalin-like peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. D. Hexum	Visiting Associate	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Majane	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The existence in brain, sympathetic ganglia and adrenal medulla of multiple forms of peptides that cross react with antibodies specific for enkephalin has been demonstrated. These enkephalin-like peptides are particularly abundant in adrenal glands where they also exhibit a considerable amount of molecular heterogeneity. One of the newly detected enkephalin-like peptides from caudate is biologically active in inhibiting the electrically induced contraction of guinea pig ileum and causing catatonia when injected intraventricularly. These effects are naloxone resistant indicating that they act on receptors different from those involved in the antinociceptive action of morphine. A similar enkephalin-like peptide also exist in adrenal medulla. This novel enkephalin-like peptide may play an important role in the adrenal function since it is a major component of the total low molecular weight enkephalin-like peptides.

PHS-6040
(Rev. 10-76)

Project Description:

It is generally recognized that tissues contain multiple molecular forms of various biologically active peptides that they store. This molecular heterogeneity includes the molecular weight and the amino acid sequence. Enkephalins are not exceptional and radioimmunoassay of tissue stores detects a heterogeneous group of peptides including the pentapeptide, enkephalin, and other higher molecular weight immunoreactive peptides. The specificity of the enkephalin antiserum which exhibits binding affinity for structurally related peptides but not for dissimilar peptide sequences suggests that the immunoreactive enkephalin-like peptides probably represent a group of peptides which includes the enkephalin sequence within their structure. In order to explore the biological properties of the newly detected enkephalin-like peptides we used a number of bioassays. One of the newly detected enkephalin-like peptides which is isolated from bovine striatum and consists of between 6 to 10 amino acid residues is found to inhibit the muscle contraction elicited by electrical stimulation of the myenteric plexus preparation of guinea pig ileum and to cause catatonia when injected intraventricularly. Catatonia fails to follow the injection of 200 μg of synthetic enkephalin or enkephalin purified with a similar procedure from bovine caudate. Both responses are not antagonized by naloxone, indicating that the biological property of the newly detected enkephalin-like peptide is different from that of enkephalins. In addition, the newly detected enkephalin-like peptide is found to be more resistant to exopeptidases in comparison to enkephalins.

The existence of multiple forms of enkephalin-like peptides has been demonstrated in brain as well as in peripheral tissues such as adrenal glands and sympathetic ganglia. The molecular heterogeneity is especially pronounced in peripheral tissues where the enkephalin often is not the predominant form. Gel filtration chromatography revealed the existence of at least three low molecular weight enkephalin like peptides in the adrenal gland. In order to explore the biological function(s) of these enkephalin-like peptides in the adrenal gland we are planning to isolate and characterize both chemically and biologically the newly detected low molecular weight enkephalin-like peptides in the adrenal gland, and to study the regulation of their secretion from this tissue.

Publications:

Yang, H.-Y. T., Hong, J. S., and Costa, E.: The search for proenkephalins: Experimental tools and status of art. In Application of RIA to Cardiovascular Medicine. Amsterdam, The Netherlands, Elsevier/North Holland Biomedical Press, in press.

Costa, E., Di Giulio, A., Fratta, W., Hong, J., and Yang, H.-Y. T.: Interactions of enkephalinergic catecholaminergic neurons in CNS and periphery. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01541-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

In vivo inactivation of enkephalins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Stine	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

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TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The exact molecular mechanisms involved in the catabolism of enkephalin are still unclear, but various reports strongly suggest that Tyr-Gly-Gly may be a major metabolite produced by the action of an exopeptidase such as carboxypeptidase or dipeptidyl carboxypeptidase. In order to test which exopeptidase is involved in the catabolism of enkephalin, we have studied the effect of dipeptidyl carboxypeptidase inhibitor, captopril, on the rate of disappearance of intraventricularly injected enkephalin and on endogenous brain enkephalin content. The drug prolongues the half life of injected enkephalin and potentiates the analgesic effect of the injected enkephalin but fails to change the content of endogenous enkephalin in either control or stressed animals. These results suggest that dipeptidyl carboxypeptidase may not be involved in the catabolism of endogenous enkephalin. We are currently studying the effect of a carboxypeptidase A inhibitor on the enkephalin content of various brain parts of normal and stressed rats.

Project Description:

Since the discovery of enkephalins in 1975, many investigators have attempted to elucidate the molecular mechanism involved in the catabolism of enkephalins. The practical aspect of such studies derives from the lack of tolerance observed following repeated stimulations of antinociceptive centers of the brain. Hence it is hoped that an inhibitor of enkephalin catabolism may cause analgesia without inducing tolerance to the antinociceptive effects as it occurs in the case of morphine and other agonists of opiate receptors. Many studies on the enkephalin metabolism have been carried out by identifying the breakdown products of [^{3}H]-enkephalin incubated with tissue extracts or injected intraventricularly. These studies fail to clarify the nature of the specific enzyme responsible for enkephalin metabolism and suggest that injected enkephalin is degraded by a number of exopeptidases including aminopeptidase, carboxypeptidase and dipeptidyl carboxypeptidase. In order to test the possibility that one of these enzymes may be the specific enzyme for the inactivation of enkephalin, we have studied the effect of the dipeptidyl carboxypeptidase inhibitor, captopril on, 1) the catabolism of intraventricularly injected [^{3}H]-enkephalin, 2) potentiation of analgesia induced by enkephalin and 3) the endogenous level of enkephalin after subjecting rats to painful stimuli. The enkephalin injected intraventricularly is degraded at a slower rate in rats pretreated with captopril (900 µg/rat). In good agreement with this observation, the analgesic effect of intraventricularly injected enkephalin is potentiated in animals pretreated with captopril. In some animals, analgesia was induced by captopril alone. However, the endogenous level of enkephalin is not affected by captopril in either normal control or foot shock stressed rats. These results suggest that captopril is effective in protecting the inactivation of injected enkephalin to some degree but does not affect the catabolism of endogenous enkephalin. Captopril, which is a potent inhibitor of angiotensin converting enzyme, inhibits also the aminopeptidase at a high dose. Thus, it is likely that the intraventricularly injected enkephalin is degraded by both aminopeptidase and dipeptidyl carboxypeptidase since a rather high dose of captopril (900 µg/rat) is required to observe its effect. The carboxypeptidase A inhibitor carbobenzoxy-D-phenylalanine has been reported to potentiate the analgesia which is induced by foot shock stress in rats. Because of this report, we are currently studying the effect of the carboxypeptidase A inhibitor on the endogenous levels of enkephalin in control and foot shock stressed rats. The purpose of this study is to examine 1) whether carboxypeptidase A is involved in the enkephalin catabolism and 2) whether enkephalin neurons are normally inactive and can be activated by painful stimuli.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01542-01 SMRP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Amino acid neurotransmitter and hippocampal function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Schmid	Visiting Fellow	SMRP	NIMH
Other:	J. L. Meek	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of the amino acid distribution in serial sections (400 μl) of hippocampus followed by lesions of granula cells and/or fimbria will be used to learn about distribution of pools of GABA, taurine, glutamate and aspartate working as putative neurotransmitters.

Project Description:

An increasing amount of evidence suggests that glutamate and aspartate may act as chemical neurotransmitters in the CNS. While the neurophysiological data are convincing the biochemical evidence remains insufficient.

A sensitive, precise and fast method for the simultaneous measurement of taurine, aspartate, glutamate, glycine, alanine and GABA was developed, utilizing high pressure liquid chromatography. The amino acids were separated on a Cation-exchange column, they were detected and quantified by measuring the fluorescence resulting from acylation with o-phthaldialdehyde. A content of about 10 pmoles can be analyzed in about forty minutes.

To learn more about the neuronal location, function and interactions of GABA, aspartate, glutamate, taurine and glycine we selected the hippocampus because of the possibility, suggested in various literature reports, that this structure may contain taurinergic, aspartergic, GABAergic and glutamatergic neurons. Moreover the cytoarchitecture of this area facilitates the study of the neuronal location of putative transmitters by allowing specific lesions of afferent pathways (fimbria, perforant afferent, cingulate afferent) or the selective stimulation of neuronal efferents to the specific cells in slices.

Since some of the amino acids that we intended to study as a putative neurotransmitters are rapidly metabolized, to avoid errors due to postmortem metabolism the rats were killed by microwave irradiation focussed to the head, which in a few seconds inactivates the brain enzymes.

The hippocampus was dissected in coronal slices 400 µ thick and changes in the steady state of the neurotransmitters were studied following surgically or chemically induced lesions. Kainic acid (KA), a very potent neurotoxic agent, which causes degeneration of neuronal perykaria while it spares glial and axons was used to induce chemical lesions.

Results

KA (2 µg) was injected into the dorsal part of the hippocampus and the animals were killed after a period of two or three days. Histological sections showed a severe degeneration of neurons in the area of KA injection.

These injection of KA caused massive degeneration of neurons at the injection site. Content of GABA, glutamic acid, aspartic acid, and taurine was severely reduced in the areas contigues to the KA injection.

In contrast, the amino acids, glycine and alanine showed no significant changes, suggesting that these amino acids are not concentrated in neurons and therefore may not function as neurotransmitters in the hippocampus.

The time course of the hippocampal changes showed that the reduction of the above amino acids was greatest after two or three days following the injection of KA. When the same amount of KA was injected into the dorsal part of the hippocampus the highest reduction could be found in the area of the injection, the amino acid content was reduced by a smaller extent in the medial part of the hippocampus while there was no change in the ventral part. This finding could be interpreted, to indicate that there are no neuronal interconnections between the dorsal and ventral hippocampus established by neurons using GABA, taurine, glutamate and aspartate as transmitters.

The analysis of the septum after injection of KA in the dorsal part of the hippocampus showed a slight increase in the GABA content. Since it is known that there is a positive feedback from hippcampus to the septum, this increase could be an index of the decrease utilization of septal GABA following the fimbria transection.

In conclusion the work up to now indicates a function of the amino acids; glutamate, aspartate and taurine beside that of GABA as neurotransmitters within the hippocampus, showing big reductions after neuronal degenration in this area. By dissecting serial cerebral section (400 μ) of hippocampus into CA_1, CA_2, CA_3 and CA_4 areas and lesioning fimbria or granule cells it is hoped to learn more about the distribution of these amino acids. Stimulation of slides with and without lesions should give evidence for the neurally mediated release of these putative neurotransmitters.

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